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Evaluation of nematicidal activity of the essential oil of *Homalomena occulta* (Lour.) Schott rhizome and its major constituents against *Meloidogyne incognita* (Kofoid and White) Chitwood

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Abstract
In a screening program for new agrochemicals from Chinese medicinal herbs and wild plants, essential oil of *Homalomena occult*a (Lour.) Schott rhizome was found to possess strong nematicidal activity against the root-knot nematodes, *Meloidogyne incognita* (Kofoid and White) Chitwood. The essential oil of *H. occulta* rhizome was extracted via hydrodistillation and investigated by gas chromatography-mass spectrometry (GC-MS). A total of 39 components of the essential oil were identified. Linalool (47.7%) was the major compound of the essential oil of *H. occulta* followed by 4-terpineol (16.5%) and α-terpineol (11.2%) and 87.3% of the total components were monoterpenoids. The essential oil possessed strong nematicidal activity against *M. incognita* with a LC_{50} value of 156.43 µg/ml. α-Terpineol and 4-terpineol exhibited toxicity against *M. incognita* with LC_{50} values of 103.41 µg/ml and 115.17 µg/ml, respectively while linalool had a LC_{50} value of 180.36 µg/ml.

Keywords: *Homalomena occulta; Meloidogyne incognita;* nematicidal activity; essential oil composition; α-terpineol; 4-terpineol; linalool.

1. Introduction
Homalomena occulta (Lour.) Schott is a perennial herb of Araceae, mainly distributed in the Southeastern and Southwestern China, e.g., Guangdong, Guangxi, and Yunnan provinces [1]. The rhizome of *H. occulta* has been used in traditional Chinese medicine for the treatment of stomach diseases and rheumatoid arthritis, and also as anti-inflammatory agent and as tonics [2]. The aqueous extract of *H. occulta* exhibited anti-histamine, anti-coagulant, anti-inflammatory and analgesic activities [3]. A series of sesquiterpenoids, triterpenoids, phenolic acids and other compounds [4-10] was obtained from organic extracts of dry rhizome or aerial parts of *H. occulta*. The phenolic acids derived from *H. occulta* exhibited BACE1 (β-secretase) inhibitory activity [9] and four of the sesquiterpenoids had a stimulative effect on proliferation and differentiation of cultured osteoblasts [7]. Composition of *H. occulta* essential oil had been widely studied [11-16]. The essential oil of *H. occulta* rhizomes showed moderate antioxidant activity [11] and insecticidal and repellency activity of the essential oil of *H. occulta* against the red flour beetles (*Tribolium castaneum*) were observed [17]. During a mass screening program for new agrochemicals from Chinese medicinal herbs and wild plants, essential oil of *H. occulta* rhizome was found to possess strong nematicidal activity against the root-knot nematodes, *Meloidogyne incognita* (Kofoid and White) Chitwood. *M. incognita* is the most economically important and widely distributed nematode throughout China and a considerable crop loss is caused by this nematode. Root-knot nematodes spend part of their life in soil either as eggs or as second-stage larvae. The latter enter the roots and establish feeding sites in susceptible hosts, inducing roots swelling with a characteristic “knotty” appearance. Root galling can drastically limits water and nutrient uptake leading to several symptoms, like malnutrition, chlorosis, and stunting, causing considerable quantitative and qualitative losses in several crop plants. A literature survey has shown that there is no report on nematicidal activity of the essential oil of *H. occulta* rhizome; thus we decided to investigate nematicidal activity of the essential oil and its main components against nematodes. Chemical composition of the essential oil was also determined.
Plant-parasitic nematodes are responsible for substantial economic loss to agricultural crops. Nematode management is generally based upon chemical treatments (soil fumigation), but

environmental concerns and governmental regulations are now resulting in a strong interest in nematicides of natural origin [18, 19]. One alternative is to screen naturally occurring compounds in plants, which are known as plant secondary compounds. Many plant constituents and metabolites including essential oils and monoterpenoids have been investigated for activity against plant-parasitic nematodes [20-30]. A series of nematicidal substances of plant origin such as triglycerides, sesquiterpenoids, alkaloids, steroids, diterpenoids, monoterpenoids and flavonoids have been identified [19]. In part, because certain plant essential oils meet the criteria of minimum risk pesticides by EPA of US [31], much effort has been focused on them and their constituents as potential sources of commercial nematode control products [32]. For example, Kim et al. [26] evaluated 28 commercial essential oils for their nematicidal activities against the pine wood nematode, *Bursaphelenchus xylophilus* and 22 pure compounds from the essential oils were also evaluated for nematicidal activity. In another report, 8 essential oils from Greek Lamiaceae species and 13 monoterpenes were screened for nematicidal activity against the root-knot nematode [21] while Echeverrigaray et al. [23] also evaluated the nematicidal activity of 22 monoterpenoids against the root-knot nematode and found that in general, compounds with hydroxyl and carbonyl groups exhibited higher nematicidal activity than other terpenoids. These results suggest that some of the essential oils tested and selected monoterpenoids are potential natural pesticides in the control of nematodes [32].

2. Materials and Methods

2.1. Chinese medicinal herb and essential oil extraction

The rhizome of *H. occulta* (10 kg) was purchased from Anguo Herb Market (Anguo, Hebei Province, China). The herb was identified, and a voucher specimen (CAU-Zhongyao-Qiannianjian-R001) was deposited at the museum of Department of Entomology, China Agricultural University. It was firstly grinded to powdered form using a grinding mill (Retsch Muhle, Germany) and subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. Essential oil was stored in airtight containers in a refrigerator at 4°C for subsequent experiments. Linalool, α-terpineol, and 4-terpineol were purchased from Sigma-Aldrich Chemical Co. (P.O.Box 14460, St. Louis, MO 63178, USA). Carbofuran was purchased from National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang 110021, China) and used as a positive control.

2.2. Nematicidal Assay

Egg masses of *M. incognita* (Kofoid and White) Chitwood obtained from tomato roots with aid of a stereomicroscope were maintained in petri dishes for 24 h in distilled H_2O for the juvenile eclosion. Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of *H. occulta* essential oil (5 concentrations) and pure compounds (5 concentrations) was prepared in H_2O solution with 1% dimethyl sulfoxide (DMSO). 20 µL portions of H_2O containing approximately 30 juveniles (J_2) were transferred to vials to which 980 µL of the solution containing essential oil or pure compounds was added. The vials were kept on a hood at 25 °C. The counting of the inactive nematodes was performed at every 24 h for 72 h. After the last counting, 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 1% DMSO in H_2O solution as control. The experiments were repeated three times.

2.3. GC-MS analysis

The essential oil of *H. occulta* rhizome 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, diluted to 1:100 in *n*-hexane) was injected with a split ratio of 1: 10. The carrier gas was helium at a 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–C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [33]. Relative percentages of the individual components of the essential oil were obtained by averaging the GC-FID peak area% reports.

2.4. Statistical analysis

The observed mortality data were corrected for control mortality using Abbott's formula [34]. The results from all the replicates were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LC_{50} values with their fiducial limits [35].

3. Results and Discussion

The yellow essential oil yield of *H. occulta* rhizome was 0.84% v/w on a dry weight basis and the density of the concentrated essential oil was 0.79 g/ml. A total of 39 components of the essential oil of *H. occulta* rhizome were identified, accounting for 98.5% of the content of the oil (Table 1). The main component was linalool (47.7%) followed by 4-terpineol (16.5%), α-terpineol (11.2%), and geraniol (3.7%). Most of the essential oil was monoterpenoids (87.3%) and only 10.9% sesquiterpenoids. The chemical composition of the essential oil was some different from that reported in other studies. For example, linalool (67.66%) was the major constituent followed by 4-terpineol (5.09%) and geraniol (2.01%) in the essential oil of *H. occult* collected from Vietnam [12] while the essential oil of *H. occult* harvested from Hunan Province, China mainly contained linalool (30.18%), torreyol (12.92%), 4-isopropyl-1-methyl-3-cyclohexen-1-ol (11.84%), α-terpineol (6.37%), *trans*-geraniol (5.11%) and spathulenol (4.64%) [13]. However, epi-α-cadinol (14.8%), α-cadinol (14.8%), α-terpineol (13.8%), linalool (11.1%), 4-terpineol (4.92%) and δ-cadinene (4.91%) were the major constituents of the essential oil of *H. occult* purchased from Hong Kong (originally produced in Guangxi Province, China) [11]. The essential oil of *H. occult* obtained by supercritical fluid CO_2 extraction contained linalool (30.53%), 1-cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl) (6.01%), linoleic acid (5.42%) and 4-(2,6,6-trimethyl-cyclohex-1-enyl)-butyric acid (5.24%) [14]. It seems that linalool is always one of main components in the essential oil of *H. occulta* rhizome in the previous reports as well as in the present work. The above findings suggest that there were great variations in chemical composition of the essential oil of *H. occulta* rhizome and it maybe due to population varieties or geographic factors as well as storage time.

Thus, for practical use, it is necessary to standardize the essential oil of *H. occulta* rhizome.

The essential oil of *H. occulta* rhizome possessed strong nematicidal activity against *M. incognita* with a LC_{50} value of 156.43 µg/ml (Table 2). Compared with the synthetic insecticide, carbofuran (LC_{50} = 72.29 µg/ml), the essential oil exhibited only half level of toxicity against *M. incognita*. Among the three main components, α-terpineol (LC_{50} = 103.41 µg/ml) and 4-terpineol (LC_{50} = 115.17 µg/ml) exhibited stronger nematicidal activity than the crude essential oil and linalool (LC_{50} = 180.36 µg/ml) against *M. incognita* (Table 2). However, all the three compounds showed weaker toxicity against the root-knot nematodes than carbofuran. Considering the positive control is synthetic insecticide, nematicidal activity of the essential oil of *H. occulta* rhizome and the three major constituents is quite promising and they showed potential to be developed as a possible natural nematicide for control of the root-knot nematodes. Moreover, for the practical application of the essential oil/constituent as a novel nematicide, further studies on the safety of the essential oil and the three constituents 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 of *Homalomena occulta* rhizomes.

Peak no.	Compounds	RI[a]	Composition (%)
1	α-Pinene[a]	939	0.1
2	Camphene	949	0.1
3	Fenchene	957	0.1
4	β-Pinene[a]	981	0.2
5	β-Myrcene[a]	991	0.4
6	δ-2-Carene	1002	0.3
7	*p*-Cymene	1020	0.1
8	1,8-Cineole[a]	1029	0.2
9	Limonene[a]	1030	0.3
10	γ-Terpinene	1057	0.4
11	*trans*-Linalool oxide	1067	1.7
12	Terpinolene	1088	0.1
13	Linalool[a]	1094	47.7
14	β-Terpineol	1147	1.7
15	4-Terpinnol[a]	1175	16.5
16	α-Terpineol[a]	1191	11.2
17	γ-Terpineol	1205	0.2
18	Nerol[a]	1232	1.4
19	Cuminal	1243	0.3
20	Carvone[a]	1250	0.3
21	Geraniol[a]	1263	3.7
22	Cuminol	1295	0.2
23	Carvacrol	1308	0.1
24	Copaene	1374	0.3
25	α-Gurjunene	1407	0.4
26	β-Caryophyllene[a]	1420	0.2
27	γ-Muurolene	1471	0.6
28	δ-Selinen	1492	0.1
29	α-Muurolene	1499	0.2
30	β-Bisabolene	1508	0.3
31	δ-Cadinene	1524	0.3
32	α-Calacorene	1543	0.5
33	Ledol	1562	0.3
34	Spathulenol	1578	0.3
35	Torreyol	1639	1.0
36	τ-Muurolol	1642	2.0
37	α-Cadinol	1653	3.0
38	Cadalene	1674	1.4
39	Phytol	2119	0.3
	Total		98.5
	Monoterpenoids		87.4
	Sesquiterpenoids		10.9
	Others		0.3

[a] RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons; [b]Identification based on comparison of LRI and spectra with authentic standards.

Table 2: Nematicidal activity of the essential oil of *H. occulta* rhizomes and its three main components against *Meloidogyne incognita*.

Treatments	Concentrations (μg/ml)	LC_{50} (μg/ml)	95% Fiducial limits	Chi-Square Tests (χ^2)
H. occulta	25.0-400.0	156.43	141.93-171.87	13.21
Linalool	80.0-860.0	180.36	173.72-197.99	9.64
α-Terpineol	40.0-672.0	103.41	93.22-122.51	13.73
4-Terpineol	40.0-672.0	115.17	14.81-127.57	14.52
Carbofuran	25.0-400.0	72.29	66.86-79.97	13.57

The mode of action of essential oils and their component against nematodes is unclear. There are few reports available now. For example, nematicidal activity of two monoterpenoids (thymol and carvacrol) might be mediated through tyramine receptor as the two compounds could trigger the signaling cascade downstream from the receptor in cells expressing wild-type but not a mutant ser-2 tyramine receptor [36]. Moreover, several constituents from the essential oils inhibited acetylcholinesterase activities of insects and nematodes [37, 38]. Constituents of the essential oils are antagonists of octopamine receptors of American cockroaches (*Periplaneta americana*) [39]. Octopamine is a neurotransmitter in insects. The involvement of essential oil components in interrupting the nematode nervous system is unclear; however, essential oils may disrupt the cell membrane of the nematode and change its permeability [20]. It is interesting to note that the main components of the essential oils that revealed nematicidal activity in this study have also been reported to have insecticidal activities [40-44]. In the previous studies, the three main components were demonstrated to exhibit toxicity against the nematodes [21]. For example, α-terpineol and 4-terpineol exhibited contact toxicity against *Bursaphelenchus xylophilus* with LC_{50} values of 3.39 mg/ml and 2.61 mg/ml, respectively [45] while linalool with a LC_{50} values of 6.44 mg/mL [46]. The three compounds significantly reduced hatching and reduced J_2 mobility of the root-knot nematode *M. incognita* at a concentration of 250 mg/l [23].

4. Conclusions

The essential oil of *H. occulta* rhizomes and the major constituents demonstrated some nematicidal activity against *M. incognita*. They showed potential to be developed as possible natural nematicide for control of the root-knot nematode but needs to be further evaluated for safety in humans and to enhance its activity.

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