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柠檬草和肿柄菊叶精油的抗线虫活性

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摘 要:柠檬草(*Cymbopogon citratus*)和肿柄菊(*Tithonia diversifolia*)为引种到我国的热带和亚热带植物。采用水蒸气蒸馏法提取柠檬草和肿柄菊叶精油,得率分别为1.02%和1.05%(w/w)。通过GC和GC-MS分析精油的组成。从柠檬草叶精油中鉴定出20个化合物,单萜和倍半萜占81.25%,精油中含量较高的化合物有:柠檬醛(49.76%)、月桂烯(8.54%)、橙花醇(5.92%)。从肿柄菊叶精油中鉴定出20个化合物,单萜和倍半萜占95.85%,精油中含量较高的化合物有:α-松油醇(20.31%)、1,8-桉树脑(14.67%)、1,7,7-三甲基二环[2.2.1]庚-2-醇(14.32%)、2,6,6-三甲基二环[3.3.1]庚-2-烯(13.51%)和1,1,4,7-四甲基十氢-1*H*-环丙并[e]薁-4-醇(7.39%)。两种精油对松材线虫、南方根结线虫、全齿复合线虫和秀丽隐杆线虫均表现较强的抑制活性。两种精油对南方根结线虫的抑制活性均最强,用柠檬草精油处理南方根结线虫24 h和48 h,半抑制浓度(IC_{50})分别为1.068 μg/mL和0.747 μg/mL;用肿柄菊精油处理南方根结线虫24 h和48 h,IC_{50}值分别为1.118 μg/mL和1.039 μg/mL。研究结果为揭示柠檬草和肿柄菊精油中的抗线虫成分,以及这两种植物精油作为抗线虫剂的开发提供了依据。

关键词:柠檬草;肿柄菊;精油;化学组成;抗线虫活性;松材线虫;南方根结线虫

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Antinematodal Activity of the Leaf Essential Oils of *Cymbopogon citratus* and *Tithonia diversifolia*

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Abstract: The leaf essential oils of lemongrass (*Cymbopogon citratus*) and Mexican sunflower (*Tithonia diversifolia*) were obtained with a yield of 1.02% (w/w) and 1.05% (w/w), respectively by hydro-distillation. The chemical components of the essential oils were investigated by GC-FID and GC-MS. Citral (49.76%), myrcene (8.54%) and nerol (5.92%) were the major compounds of the 20 identified components which accounted for 98.00% of the total lemongrass oil where 81.25% belonged to monoterpenoids and sesquiterpenoids. α-Terpineol (20.31%), 1,8-cineole (14.67%), 1,7,7-trimethylbicyclo[2.1.1]heptan-2-ol (14.32%), 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (13.51%) and 1,1,4,7-tetramethyldecahydro-1*H*-cyclopropa[e]azulene-4-ol (7.39%) were the major compounds of the 20 identified components which accounted for 98.49% of the total Mexican sunflower oil where 95.85% belonged to monoterpenoids and sesquiterpenoids. Both essential oils had similar antinematodal activity with a broad spectrum. Among the four tested nematodes (*Bursaphelenchus xylophilus*; *Meloidogyne incognita*, *Panagrellus redivivus* and *Caenorhabditis elegans*), both oils showed the strongest inhibition on root knot nematode (*M. incognita*) with the IC_{50} values of 1.068 μg/mL and 0.747 μg/mL respectively at 24 h and 48 h of treatment for the lemongrass oil, and 1.118 μg/mL and 1.039 μg/mL respectively at 24 h and 48 h of treatment for the Mexican sunflower oil. The results provided supporting data for activity-guided fractionation of the antinematodal components from these two oils as well as future development of the essential oils as the antinematodal agents.

Key words: *Cymbopogon citratus*; *Tithonia diversifolia*; essential oil; chemical composition; antinematodal activity; *Bursaphelenchus xylophilus*; *Meloidogyne incognita*

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Introduction

Plant parasitic nematodes (*e.g.*, root-knot nematodes

and pine wood nematode), which constitute one of the most important pest species groups affecting a wide variety of plants, cause significant damage to a broad range of vegetables, agricultural crops and forest trees[1]. Root-knot nematodes belong to the genus *Meloidogyne* which mainly damage vegetable crops [2]. Pine wood nematode (*Bursaphelenchus xylophilus*) has been the most serious forest pathogen which devastates pine trees [3]. For decades, the control of nematodes has relied heavily on the synthetic nematicides which have resulted in significant environmental pollution and resistance. Hence it is necessary to continuously search for environment-friendly and efficacious nematicides[4]. Many plants have been reported to possess antinematodal activity, and a series of antinematodal substances of plant origin such as terpenoids, alkaloids, flavonoids and polythiophenes have been identified[5]. Furthermore, the use of plant products is one of the promising methods for nematode control. They are cheap, easy to apply, produce no pollution hazards, and have the capacity to structurally and nutritionally improve soil health[6]. Plant essential oils and their components possess multi-biological activities such as antimicrobial, insecticidal, antiviral and antinematodal properties. They have received much attention to prevent plant and animal diseases [7-9].

Lemongrass (*Cymbopogon citratus*) (Gramineae) is indigenous medicinal plant in tropical and subtropical areas of Asia for the treatment of various diseases such as stomachache and gastric ulcer, and is cultivated in South and Central America, Africa, and other tropical and subtropical areas[10]. The essential oil from lemongrass has been reported to have a variety of biological activities such as antifungal [11], antibacterial [12], phytotoxic effects on the germination and seedling growth of bamyardgrass (*Echinochloa crus-galli*)[13], anti-*Leishmania* activity [14].

Mexican sunflower (*Tithonia diversifolia*) (Compositae) is a traditional medicinal plant of Central American countries for the treatment of malaria, fever and wound[15]. It mainly contains sesquiterpene lactones, monoterpenes, and flavonoids to have phytotoxic, leishmanicidal, and anti-hyperglycemic activities[15-18]. The essential oil of Mexican sunflower has been characterized for its composition[19], and was screened to show its allelopathic effect[20].

Both lemongrass and Mexican sunflower were brought to China at the end of the last century[20]. However, no reports have documented on the chemical components and antinematodal activity of the leaf essential oils of these two plants from China. The aim of the present study was to analyze the chemical composition of the leaf essential oils of lemongrass and Mexican sunflower from China by GC-MS as well as to evaluate their *in vitro* antinematodal activity for future development of the essential oils as antinematodal agents.

Materials and Methods

Plant materials

The leaves of lemongrass (*Cymbopogon citratus*) and Mexican sunflower (*Tithonia diversifolia*) were collected in Kunming of China in June 2011. They were dried in the shade at room temperature. The plant taxonomical identifications were done by Dr. Zhilong Liu of China Agricultural University, where the voucher specimens were deposited.

Preparation of the essential oils

The dry leaves (1.0 kg for each species) of lemongrass and Mexican sunflower were separately submitted to hydro-distillation in a Clevenger-type apparatus at 100 ℃ for 4 h. The distilled oil was extracted with diethyl ether and dried over anhydrous sodium sulfate. After filtration, the yields of the essential oils were 10.2 g (1.02%, w/w) for lemongrass and 10.5 g (1.05%, w/w) for Mexican sunflower. The oils were then preserved in a sealed dark glass vial at 4 ℃ until required.

Oil analysis

The composition of each leaf essential oil was determined using GC-FID and GC/MS. The same column and analysis conditions were used for both GC-FID and GC/MS. An Agilent 6890N Network GC system was equipped with an HP-5MS column [30 m × 0.25 mm (5%-phenyl)-methylpolysiloxane capillary column, film thickness 0.25 μm], a split-splitless injector heated at 250 ℃ and a flame ionization detector (FID) at

240 ℃. The oven temperature was programmed as follows: initial temperature 50 ℃ for 1.50 min, increased by 10 ℃/min up to 180 ℃, 2 min at 180 ℃, and then increased by 6 ℃/min up to 280 ℃, 10 min at 280 ℃. Helium (99.999%) was used as carrier gas at a flow rate of 1.0 mL/min. The injection volume was 1.0 μL (split ratio 1∶20). GC-MS analyses were performed using an Agilent 6890N Network GC system with an Agilent 5973 Network mass selective detector, mass spectrometer in EI mode at 70 eV in m/z range 10-550 amu.

The components were identified by comparison of their mass spectra with NIST 2002 library data of the GC-MS system, as well as by comparison of their retention indices (RI) with the relevant literature data [12,13,19-22]. The relative amount (RA) of each individual component of the essential oil was expressed as the percentage of the peak area relative to the total peak area. RI value of each component was determined relative to the retention times (RT) of a series of C_8-C_{40} *n*-alkanes with linear interpolation on the HP-5MS column[23].

Culture of the nematodes

The pinewood nematode (*B. xylophilus*) was kindly supplied by Dr. Bingyan Xie from the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences. The fungus *Botrytis cinerea* was cultured on potato dextrose agar (PDA) plate at 25 ℃ in darkness. When the fungus was fully grown, the plate was inoculated with the pinewood nematodes, and then cultured until the fungal mycelia had been completely consumed.

The root-knot nematode *M. incognita*, which was kindly supplied by Dr. Heng Jian from the College of Plant Protection, China Agricultural University, was cultured on *Ipomoea aquatica* under greenhouse conditions to obtain fresh egg masses. Fresh eggs were then kept in water for egg hatching. The second stage juveniles (J2s) that emerged from the eggs after 48 h were incubated at 30 ℃ and were used for nematicidal assay.

The nematode *Panagrellus redivivus*, which was kindly supplied by Prof. Keqin Zhang at the Laboratory for Conservation and Utilization of Bio-Resources, Yunnan University, was cultured on oatmeal agar plate at 25 ℃ in darkness for 3-5 days.

The nematode *Caenorhabditis elegans* was kindly supplied by Dr. Chongling Yang at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. The nematode was inoculated on Luria-Bertani (LB) agar plate where the bacterium *Escherichia coli* was cultured previously for 2-3 days. After another 3-5 days cultivation, the propagated nematodes were ready for the assay.

Antinematodal activity

Each essential oil (5.0 mg) was dissolved in 1.0 mL of the mixture of ethanol-Tween-20 (9∶1, v/v) to obtain the initial stock solution at 5 mg/mL, which was further diluted to 6.25, 12.5, 25, 50, 100, 125 and 250 μg/mL. The test nematode solution (90 μL containing 40 to 50 nematodes) was added into each well of the sterile 96-well microplate, and then 10 μL of sample stock solution was added into each well and mixed thoroughly. 10 μL of the mixture of ethanol-Tween-20 (9∶1, v/v) was used as the negative control. Five replicates were carried out for each treatment, and the experiments were repeated thrice. Dead/paralysis and active nematodes were counted after 12, 24 and 48 h, respectively. The nematodes were considered to be dead when they did not move to physical stimuli with a fine needle[24,25]. The mean percentage of mortality was then calculated. Avermectin, which was kindly provided by Dr. Shankui Yuan at the Institute for the Control of Agrochemicals, Chinese Ministry of Agriculture, was used as the positive control with the purity of 97.2%.

Data analysis

The mortality (%) was adjusted according to the method of Ntalli by eliminating the natural death/paralysis in negative control [26]. To describe the antinematodal effects of the essential samples against the nematodes, the median antinematodal concentration (IC_{50}) values were calculated using the linear relation between the inhibitory probability and concentration logarithm according to the method of Sakuma [27].

Results and Discussion

Essential oil analysis

After extraction, the yields (w/w) of the leaf essential

oils of lemongrass (*C. citratus*) and Mexican sunflower (*T. diversifolia*) were calculated as 1.02% and 1.05%, respectively. The oils were analyzed for their chemical composition by GC and GC-MS. Twenty compounds were identified which accounted for 98.00% of the total lemongrass oil (Table 1). The lemongrass oil was dominated by monoterpenoids and sesquiterpenoids which accounted for 81.25% of the total oil and characterized by a high percentage of citral (49.76%), myrcene (8.54%) and nerol (5.92%). The chemical profile of the lemongrass oil in this study was similar to those of the previous reports only a few components were different[12,13,21]. Similarly, α-terpineol (20.31%), 1,8-cineole (14.67%), 1,7,7-trimethylbicyclo[2.1.1]heptan-2-ol (14.32%), 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (13.51%) and 1,1,4,7-tetramethyldecahydro-1*H*-cyclopropa[e]azulene-4-ol (7.39%) were the major compounds of the 20 identified components which accounted for 98.49% of the total Mexican sunflower oil where 95.85% belonged to monoterpenoids and sesquiterpenoids (Table 2). The chemical profile of the leaf essential oil of Mexican sunflower in

Table 1 Chemical composition of the leaf essential oil from lemongrass

Compound[a]	Molecular formula	RI[b]	RA(%)[c]
6-Methyl-5-hepten-2-one	$C_8H_{14}O$	974	1.64
Myrcene = 7-Methyl-3-methyleneocta-1,6-diene	$C_{10}H_{16}$	977	8.54
Melonal = 2,6-Dimethyl-5-hepten-1-al	$C_9H_{16}O$	1066	0.85
Seudenone = 3-Methyl-2-cyclohexen-1-one	$C_7H_{10}O$	1072	1.24
Linalool = 3,7-Dimethylocta-1,6-dien-3-ol	$C_{10}H_{18}O$	1120	3.97
(*E*)-3,3-Dimethylhepta-1,5-diene	C_9H_{16}	1170	1.77
2,6,6-Trimethylcyclohexa-1,3-dienecarbaldehyde	$C_{10}H_{14}O$	1180	1.23
(*E*)-2-(2-Methylpropylidene) cyclohexanone	$C_{10}H_{16}O$	1206	5.42
Nerol = (*E*)-3,7-Dimethylocta-2,6-dien-1-ol	$C_{10}H_{18}O$	1283	5.92
Citral = (*E*)-3,7-Dimethylocta-2,6-dienal	$C_{10}H_{16}O$	1303	49.76
Undecan-2-one	$C_{11}H_{22}O$	1318	1.75
3,7-Dimethylocta-1,6-dien-3-yl formate	$C_{11}H_{18}O_2$	1327	0.79
7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalen-1-ol	$C_{15}H_{26}O$	1659	5.39
4-Isopropyl-1-methyl-6-methylene-1,2,3,5,6,7,8,8a-octahydronaphthalene	$C_{15}H_{22}$	1678	1.33
10-Epicadinol = 4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-ol	$C_{15}H_{26}O$	1692	2.41
1,1,3a,7-Tetramethyl-1a,2,3,3a,4,5,6,7b-octahydro-1*H*-cyclopropa[*a*]naphthalene	$C_{15}H_{25}$	1700	1.95
(3*E*,6*Z*)-6-(3,3-Dimethyloxiran-2-ylidene)-5,5-dimethylhex-3-en-2-one	$C_{12}H_{18}O$	1761	1.94
1-(4-Hydroxy-7-isopropyl-4-methyl-octahyydro-1*H*-inden-1-yl) ethanone	$C_{15}H_{26}O_2$	1777	0.75
3,5,5-Trimethylcyclopent-2-enone	$C_8H_{12}O$	1942	0.62
3,4,4-Trimethylcyclopent-2-enone	$C_8H_{12}O$	1946	0.73
Total			98.00
Monoterpene hydrocarbons			8.54
Oxygenated monoterpenes			60.88
Sesquiterpene hydrocarbons			3.28
Oxygenated sesquiterpenes			8.55
Others			16.75

[a]: The identified constituents were listed in their order of elution. [b]: RI indicated the retention indices calculated against C_8-C_{40} *n*-alkanes on the HP-5MS column. [c]: RA indicated relative amount (peak area relative to the total peak area).

Table 2 Chemical composition of the leaf essential oil from *T. diversifolia*

Compound[a]	Molecular formula	RI[b]	RA(%)[c]
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	917	13.51
o-Cymene	$C_{10}H_{14}$	1022	2.29
1,8-Cineole = 1,3,3-Trimethyl-2-oxa-bicyclo[2.2.2]octane	$C_{10}H_{18}O$	1034	14.67
1-Isopropyl-4-methylcyclohexa-1,4-diene	$C_{10}H_{16}$	1072	0.57
2-(5-Methyl-5-vinyl-tetrahydrofuran-2-yl) propan-2-ol	$C_{10}H_{18}O_2$	1093	1.36
6-Methyl-2-(oxiran-2-yl) hept-5-en-2-ol	$C_{10}H_{18}O_2$	1111	1.54
Linalool = 3,7-Dimethylocta-1,6-dien-3-ol	$C_{10}H_{18}O$	1121	0.77
1,3,3-Trimethylbicyclo[2.1.1]heptan-2-ol	$C_{10}H_{18}O$	1141	4.64
2(2,2,3-Trimethylcyclopent-3-enyl) acetaldehyde	$C_{10}H_{16}O$	1153	2.64
6,6-Diethyl-2-methylenebicyclo[3.1.1]heptan-3-ol	$C_{10}H_{16}O$	1168	5.11
1,7,7-Trimethylbicyclo[2.1.1]heptan-2-ol	$C_{10}H_{18}O$	1194	14.32
1-Isopropyl-4-methylcyclohex-3-enol	$C_{10}H_{18}O$	1203	1.82
α-Terpineol = 2-(4-Methyl-1-cyclohex-3-enyl) propan-2-ol	$C_{10}H_{18}O$	1220	20.31
(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl) methanol	$C_{10}H_{16}O$	1225	0.36
2-Methyl-5-(prop-1-en-2-yl) cyclohex-2-enol	$C_{10}H_{16}O$	1245	1.48
Carvacrol = 5-Isopropyl-2-methylphenol	$C_{10}H_{14}O$	1306	0.44
Isoledene = 1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7b-octahydro-1-*H*-cyclopropa[e]azulene	$C_{15}H_{24}$	1605	0.66
1,1,7-Trimethyl-4-methylene-decahydro-1*H*-cyclopropa[e]azulen-7-ol	$C_{15}H_{24}O$	1615	3.38
1,1,4,7-Tetramethyldecahydro-1*H*-cyclopropa[e]azulene-4-ol	$C_{15}H_{26}O$	1622	7.39
2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphtalen-2-yl) propan-2-ol	$C_{15}H_{26}O$	1689	1.23
Total			98.49
Monoterpene hydrocarbons			16.37
Oxygenated monoterpenes			66.82
Sesquiterpene hydrocarbons			0.66
Oxygenated sesquiterpenes			12.00
Others			2.64

[a]: The identified constituents were listed in their order of elution. [b]: RI indicated the retention indices calculated against C_8-C_{40} *n*-alkanes on the HP-5MS column. [c]: RA indicated relative amount (peak area relative to the total peak area).

this study was similar to those of the previous reports though a few components were different from each other [19-22]. The reasons for this phenomenon may be different geographical environments, growth seasons and age of the plant, in addition to the method of oil preparation [28].

Antinematodal activity

The antinematodal activity of the leaf essential oils of lemongrass and Mexican sunflower was evaluated against 4 representative nematodes (*i. e.*, *Bursaphelenchus xylophilus*; *Meloidogyne incognita*, *Panagrellus redivivus* and *Caenorhabditis elegans*). Its potency was assessed quantitatively by IC_{50} values which were shown in Table 3. Both essential oils had the similar antinematodal activity with a broad spectrum. Among the test nematodes, *M. incognita* was the most sensitive nematode to the oils with the IC_{50} values of 1.068 μg/mL and 0.747 μg/mL respectively at 24 h and 48 h of treatment for the lemongrass oil, and 1.118 μg/mL and 1.039 μg/mL respectively at 24 h and 48 h of treatment for the Mexican sunflower oil.

Table 3 Antinematodal activity of the leaf essential oils of lemongrass and Mexican sunflower

Tested nematode	Period of treatment (h)	IC_{50} (μg/mL)		
		Leaf essential oil of lemongrass	Leaf essential oil of *T. diversifolia*	CK$^+$ (Avermectin)
B. xylophilus	12	1.420	3.884	2.103
	24	1.347	3.350	1.568
	48	1.280	1.526	0.871
M. incognita	12	1.220	1.646	1.877
	24	1.068	1.118	1.547
	48	0.747	1.039	1.102
P. redivivus	12	1.885	1.776	1.814
	24	1.521	1.410	0.976
	48	1.261	1.053	0.781
C. elegans	12	2.383	1.836	1.733
	24	1.507	1.493	1.542
	48	1.174	1.230	1.448

Conclusion

We reported the chemical composition of the leaf essential oils of lemongrass (*C. citratus*) and Mexican sunflower (*T. diversifolia*) from China along with their antinematodal activity in this study. Both essential oils were dominated by monoterpenoids and sesquiterpenoids which accounted for 81.25% of the total lemongrass oil and 95.85% of the total Mexican sunflower oil. Citral (49.76%), myrcene (8.54%) and nerol (5.92%) were the major compounds of the 20 identified components in the lemongrass oil. α-Terpineol (20.31%), 1,8-cineole (14.67%), 1,7,7-trimethylbicyclo[2.1.1]heptan-2-ol (14.32%), 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (13.51%) and 1,1,4,7-tetramethyldecahydro-1H-cyclopropa[e]azulene-4-ol (7.39%) were the major compounds of the 20 identified components in the Mexican sunflower oil. Both essential oils had similar antinematodal activity with a broad spectrum. They showed the strongest inhibition on root-knot nematode (*M. incognita*) with the IC_{50} values of 1.068 μg/mL and 0.747 μg/mL respectively at 24 h and 48 h of treatment for the lemongrass oil, and 1.118 μg/mL and 1.039 μg/mL respectively at 24 h and 48 h of treatment for the Mexican sunflower oil. Antinematodal activity of the leaf essential oils may be due to the fact that the oil had abundant monoterpenoids and sesquiterpenoids that should be further studied. Biological properties of the essential oils are of great interest in agriculture, food, cosmetic and pharmaceutical industries[7-9]. The present study provided additional data supporting the future utilization and development of lemongrass and Mexican sunflower essential oils as the antinematodal agents. The underlying antinematodal mechanism of these two oil as well as their active components need to be further studied and clarified.

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