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Original Research Article

Chemical composition and insecticidal properties of essential oil from aerial parts of Mosla soochowensis against two grain storage insects

Xu-bo Chen, Rui Chen, Zheng-rong Luo*
College of Ecology, Lishui University, No. 1 Xueyuan Road, Lishui City, Zhejiang Province, 323000, China

For correspondence: **Email: extra@lsu.edu.cn; **Tel:** +86-578-2271308; **Fax:** +86-578-2271308*

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Abstract

Purpose: *To determine the insecticidal properties of essential oil from Mosla soochowensis aerial parts against two insect pests, Sitophilus zeamais and Tribolium castaneum.*
Methods: *Hydro-distillation of M. soochowensis was used to extract the essential oil. Gas chromatography/mass spectrometry (GC/MS) analysis was performed, and the contact (topical application) and fumigant toxicity (sealed space) of the essential oil were evaluated.*
Results: *Thirty-nine chemical compounds were identified by GC-MS analysis of M. soochowensis essential oil. The major components are β-caryophyllene (12.82 %), spatulenol (6.34 %), β-eudesmol (6.26 %), carvone (6.12 %), α-thujone (5.12 %), γ-eudesmol (4.86 %), α-cedrol (4.23 %), and α-caryophyllene (4.04 %). The plant essential oil exerted contact toxicity against adults of S. zeamais and T. castaneum (median lethal concentration (LC_{50}), 25.45 and 10.23 µg/adult, respectively). Moreover, the essential oil exhibited pronounced fumigant toxicity towards adults of both species (LC_{50} 12.19 and 10.26 mg/L air, respectively).*
Conclusion: *These results show that M. soochowensis essential oil can be used in development of safer and more natural and effective fumigants/insecticides for stored products.*

Keywords: *Mosla soochowensis, Contact toxicity, Sitophilus zeamais, Fumigant, Insecticide, Essential oil, Tribolium castaneum*

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INTRODUCTION

Contamination and destruction of stored food products by insects is a major problem for manufacturers and consumers, as the cost of such events runs to millions of dollars annually. In China, *Sitophilus zeamais* (Motsch.), commonly known as the maize weevil, and *Tribolium castaneum* (Herbst), commonly called the red flour beetle, have destructive effects on stored grain products [1], and infestations are typically controlled by fumigation techniques [2]. However, excessive use of synthetic fumigants and insecticides has several detrimental effects, including but are not limited to insecticide resistance, environmental contamination, and damage to human health and other non-target organisms [2,3]. Research into alternative treatments has shown that essential oils and their constituents possess insecticidal properties against many stored-product insect pests [4-6]; therefore, these may be alternatives to currently used fumigants and insecticides.

Mosla soochowensis Matsuda (Family: Lamiaceae) is an annual plant distributed predominantly in Anhui, Jiangsu, Jiangxi, and Zhejiang Provinces in China [7]. The aerial parts of *M. soochowensis* are applied in Chinese folk medicine to cure the common cold, tonsillitis, abdominal pain, and heartburn, and are used in ointments to treat insect and centipede bites [7]. Previous phytochemical studies on *M. soochowensis* identified several flavonoids [8,9] and the chemical composition of its essential oil [10,11]. Nevertheless, a literature search revealed that the insecticidal effects of *M. soochowensis* essential oil on grain insects have not been reported. Therefore, this study aimed to determine the chemical composition of *M. soochowensis* and the effects of its essential oil on two insect species.

EXPERIMENTAL

Plant material and extraction of essential oil

First, in August 2013, we gathered 5 kg of fresh *M. soochowensis* aerial parts at the flowering stage from Lishui (27.54 °N, 119.20 °E), located in Zhejiang Province, China. Dr. Wang from Lishui Academy of Forestry, Zhejiang, assisted with identification, A voucher specimen (category no. lsxy2013008) was kept at the herbarium of College of Ecology, Lishui University, Zhejiang, China. Samples were cut into pieces with pruning shears, and 900 g was transferred to three L-round-bottom flasks, each containing 1500 mL of tap water. The mixtures were then boiled for 6–8 hours, and the steam distilled. Because the essential oil is highly volatile, it was collected in a flask, separated from the aqueous layer using *n*-hexane in a separation funnel, and stored at 4 °C. Na_2SO_4 was used to dry the extract, and the solvent was removed using a vacuum rotary evaporator.

Analysis of the essential oil

M. soochowensis essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS) using a mass selective detector (Agilent 5973 N) and a non-polar HP-5ms capillary column of 30 m × 0.25 mm (film thickness, 0.25 µm). The column temperature program was as follows: hold at 60 °C for 1 min; increase to 180 °C at 10 °C per min; hold at 180 °C for 1 min; increase to 280 °C at 20 °C per min; and hold at 280 °C for 15 min. The injector temperature was 270 °C. Samples (1 µL were diluted 1:100 portion with acetone) were instilled with 1:10 split ratio. Helium at a flow rate of 1.0 mL per min was used as the carrier gas. Spectra were scanned from 20 to 550 *m/z* at 2 scans per second. Constituents were identified based on retention indices in the literature or data from our laboratory. The retention indices were estimated under identical operating conditions using a homologous series of *n*-alkanes (C_8–C_{24}). Further identification was performed by comparing the mass spectra with those in the NIST 05 and Wiley 275 libraries or in the literature [12]. Relative percentages were calculated based on GC peak areas, and no correction factor was applied.

Insects

The insects used in this study were from laboratory-reared colonies maintained for ≥10 years. *T. castaneum* adults were kept on wheat flour blended with yeast (10:1, w/w) at 29 –30 °C and 70 – 80 % relative humidity, while *S. zeamais* adults were reared on whole hard wheat (12 – 13 % moisture) under the same conditions. Adults insects used in this study had been growing for 2 weeks. All containers and petri dishes used were designed to prevent escape by the insects, and were coated with polytetrafluoroethylene (Fluon, Blades Biological, UK).

Test of contact toxicity

Liu and Ho [13] proposed a method for determining the contact toxicity of *M. soochowensis* essential oil. First, essential oil concentrations were evaluated. *n*-Hexane was used to serially dilute *M. soochowensis* essential oil to 3.5 – 10.0 % v/w in six steps. A Burkard hand micro-applicator (Burkard Scientific, Uxbridge, UK) was used to apply 0.5 µL aliquots to the dorsal thorax of the insects. Ten treated insects were transferred to a glass bottle (25 mL) in five replicates. *n*-Hexane was used as a negative control, and pyrethrum extract (25 % pyrethrin I and pyrethrin II, Fluka Chemie AG, Switzerland) as a positive control. The treated and control insects were kept in incubators (29 – 30 °C, 70 – 80 % relative humidity) for 24 h without a food supply, and mortality was monitored. Insects were considered dead if they did not react to a gentle touch with a dissecting needle or did not walk during a 3 min examination.

Fumigant toxicity bioassay

Essential oil fumigant toxicity was confirmed using the method of Liu and Ho [13], with minor modifications. To determine suitable test concentrations of the essential oil, several range-finding studies were conducted. *n*-Hexane was used to prepare six *M. soochowensis* essential oil solutions (3.0 – 15.0 % v/v) using Whatman filter paper (2 cm diameter) and a screw-capped glass vial (2.5 cm diameter, 5.5 cm height, 24 mL volume). An aliquot of essential oil solution (10 μL) was dropped onto filter paper in the cap of a glass vial. The cap was then replaced on the glass vial, which contained 10 unsexed insects, and closed to form an airtight chamber. *n*-Hexane was used as a control. The treated and control insects were kept in incubators (29 – 30 °C, 70 – 80 % relative humidity) for 24 h without a food supply, and mortality was monitored. Insects were considered dead if they did not react to a gentle touch with a dissecting needle or did not walk during a 3 min examination.

Statistical analysis

Abbott's formula was applied to estimate corrected percent mortality. The PriProbit software ver. 1.6.3 was used to determine LD_{50} and LC_{50} values and their 95 % confidence intervals [14]. Samples with non-overlapping 95 % fiducial limits were regarded as significantly different.

RESULTS

The yellow yield of the essential oil was 0.05 % (v/w, based on a fresh sample), and the density of the concentrated essential oil was 0.91 g/mL. Thirty-nine major components (98.40 % of the total; Table 1) were quantified. The major chemical constituents were β-caryophyllene (12.82 %), spatulenol (6.34 %), β-eudesmol (6.26 %), carvone (6.12 %), α-thujone (5.12 %), γ-eudesmol (4.86 %), α-cedrol (4.23 %), and α-caryophyllene (4.04 %) (Table 1). Sixteen constituents were sesquiterpenoids (61.23 % of the total), and 15 were monoterpenoids (28.65 %).

The *M. soochowensis* essential oil displayed contact toxicity against *S. zeamais* and *T. castaneum* adults (LC_{50} values, 25.45 and 10.23 μg/adult, respectively; Table 2). It also showed pronounced fumigant toxicity against *S. zeamais* and *T. castaneum* adults (LC_{50} values, 12.19 and 10.26 mg/L air, respectively; Table 2).

DISCUSSION

GC and GC-MS analyses revealed that β-caryophyllene, spatulenol, β-eudesmol, carvone, and α-thujone were the major chemical constituents of the essential oil of the aerial parts of *M. soochowensis*. This differs from previous reports. The major components of *M. soochowensis* essential oil from Jiangsu Province, China, were carvacrol (44.66 %), ρ-cymene (16.23 %), γ-terpinene (7.13 %), 1,8-cineole (5.76 %), and thymyl acetate (5.20 %) [11].

However, the major constituents of the essential oil of the same plant from Zhejiang Province, China, were methyl eugenol (40.40 %), nerolidene (11.00 %), dihydrocarvone (8.21 %), bornene (8.02 %), and β-thujone (7.31 %) [10]. This suggests that the oil composition of these plants differs according to location and growth stage [10,11]. Thus, the composition of this essential oil must be standardized prior to its commercial use.

The *M. soochowensis* essential oil showed significant contact toxicity against both insect species. However, the toxic effect was weaker than that for the positive control (Table 2).

Methyl bromide (MeBr) showed pronounced fumigant toxicity against both species (LC_{50} 0.67 mg/L for *S. zeamais* and 1.75 mg/L for *T. castaneum*) [13]. Compared with MeBr, the fumigant toxicity of the essential oil against maize weevil and red flour beetle was 18- and 6-fold lower, respectively.

However, *M. soochowensis* essential oil had similar or greater fumigant toxic effects on *S. zeamais* adults compared with other essential oils, such as those of *Artemisia lavandulaefolia*, *A. sieversiana* [15], *A. vestita* [16], *Glycosmis parviflora* [17], *Illicium pachyphyllum*, *I. simonsii* [18,19], *Mallotus apelta* [20], *Ostericum grosseserratum* and *O. sieboldii* [21,22]. However, the fumigant toxic effect of the essential oil against *S. zeamais* adults was weaker than that of essential oil from *Blumea balsamifera* leaves [23].

CONCLUSION

The findings suggest that *M. soochowensis* essential oil exerts contact and fumigant toxic effects on two species of grain insect, suggesting its potential as a natural fumigant for stored products. Development of viable alternatives to commercially used, synthetic compounds is important because of the spread of resistance to these pesticides and their environmental toxicity.

To best of our knowledge no study has assessed the effect of *M. soochowensis* essential oil on human health. However, because aerial parts of this plant are consumed in China as a traditional medicine, the threat to human health is likely low. Further work should include evaluation of its non-specific toxic effects.

Table 1: Major compounds of *Mosla soochowensis* essential oil

Peak no.	Compound	Retention index	(%)
	Monoterpenoids		28.65
1	α-Pinene[**]	931	0.83
2	Sabinene	975	2.56
3	β-Pinene[**]	981	1.15
4	o-Cymene[**]	1020	0.94
5	d-Limonene[**]	1027	1.68
6	1,8-Cineole[**]	1031	2.99
7	Linalool[**]	1097	0.17
8	**α-Thujone**	**1105**	**5.21**
9	*cis*-Carveol	1222	0.13
10	Thymol methyl ether	1235	0.39
11	**Carvone**[**]	**1238**	**6.12**
12	Thymol[**]	1294	3.72
13	Carvacrol	1304	1.58
14	Thymol acetate	1362	0.66
15	Carvacrol acetate	1367	0.52
	Sesquiterpenoids		61.23
16	Copaene	1375	3.72
17	α-Bourbonene	1409	1.48
18	β-Ylangene	1423	0.99
19	**β-Caryophyllene**[**]	**1428**	**12.82**
20	α-Bergamotene	1430	2.83
21	α-Caryophyllene[**]	1456	4.04
22	*trans*-α-Farnesene	1486	1.12
23	β-Bisabolene	1506	3.63
24	δ-Cadinene[**]	1523	1.81
25	β-Sesquiphellandrene	1525	1.03
26	**Spathulenol**[**]	**1578**	**6.34**
27	Caryophyllene oxide[**]	1584	3.56
28	α-Cedrol	1598	4.23
29	γ-Eudesmol	1621	4.86
30	**β-Eudesmol**[**]	**1650**	**6.26**
31	β-Bisabolol	1673	2.51
	Phenylpropanoids		7.71
32	Methyl eugenol[**]	1403	1.82
33	Myristicin[**]	1513	2.18
34	Elemicin[**]	1558	0.41
35	Asarone[**]	1645	1.58
36	Apiole	1682	1.72
	Others		0.66
37	Benzaldehyde	960	0.46
38	Acetophenone[**]	1065	0.21
39	Octyl acetate	1215	0.14
	Total identified		**98.40**

**RI, retention index; [**]Identification by co-injection of authentic compounds*

Table 2: Contact and fumigant toxicity of *Mosla soochowensis* essential oil against adults of *S. zeamais* (SZ) and *T. castaneum* (TC)

Toxicity	Insect	Treatment	LC_{50} (95% CI*)	Slope ± SD	Chi-squared (χ^2)
Contact toxicity (μg/adult)	SZ	*M. soochowensis*	25.45 (23.13–26.92)	3.08 ± 0.29	13.43[a]
		Pyrethrum extract	4.29 (3.86–4.72)	-	-
	TC	*M. soochowensis*	10.23 (9.34–11.09)	3.23 ± 0.31	10.11[a]
		Pyrethrum extract	0.36 (0.32–0.41)	-	-
Fumigant (mg/L)	SZ	*M. soochowensis*	12.19 (12.04–13.29)	3.45 ± 0.32	11.27[a]
		MeBr[**]	0.67	-	-
	TC	*M. soochowensis*	10.26 (9.32–11.26)	3.18 ± 0.28	9.32[a]
		MeBr[**]	1.74	-	-

[**]Liu and Ho [13]; [a]$p < 0.05$

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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