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Original Research Article

Gas Chromatography-Mass Spectrometric Analysis and Insecticidal Activity of Essential Oil of Aerial Parts of *Mallotus apelta* (Lour.) Muell.-Arg. (Euphorbiaceae)

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Abstract

Purpose: *To investigate the chemical composition and insecticidal activity of the essential oil of the aerial parts of Mallotus apelta against maize weevils, Sitophilus zeamais and booklice, Liposcelis bostrychophila.*
Methods: *Water-distilled essential oil of M. apelta aerial parts was analyzed by gas chromatography/mass spectrometric (GC/MS) to determine its composition. Insecticidal activities of the essential oil were measured by using topical application/ impregnated filter paper and seal-spaced fumigation.*
Results: *Thirty-six compounds, accounting for 97.75 % of the oil, were identified. The main compounds found were β-eudesmol (18.65 %), β-caryophyllene (9.83%), β-selinene (6.55 %), caryophyllene oxide (6.29 %), bornyl acetate (6.07%), γ-eudesmol (5.40 %) and α-selinene (5.06 %). The essential oil showed contact toxicity against adult maize weevils L. bostrychophila with LD_{50} (lethal dosage, 50 %) value of 46.69 µg/adult and 211.02 µg/cm, respectively. The essential oil also exhibited fumigant toxicity against adult S. zeamais and L. bostrychophila with a LC_{50} (median lethal concentration) value of 48.42 and 3.21 mg/l, respectively.*
Conclusion: *The study indicates that the essential oil of M. apelta has the potential to be developed into a natural fumigant/insecticide for the control of stored product insects.*

Keywords: *Mallotus apelta, Sitophilus zeamais, Liposcelis bostrychophila, Contact toxicity, Fumigant, Essential oil*

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INTRODUCTION

Mallotus apelta (Lour.) Muell.-Arg. (Family: Euphorbiaceae) are shrubs or small trees with 1-6 m tall and distributed widely in south China and Vietnam [1]. Roots of *M. apelta* have been used in traditional Chinese medicine for the treatments of chronic hepatitis, white blood and enteritis [2]. Phytochemical analyses of *M. apelta* lead to the isolation of a number of alkaloids, flavonoids, cumarino-lignoids, sesquiterpenoids, cembrane diterpenoids, triterpenoids, steroids, and benzopyranoids [3-7].

However, the constituents of the essential oil derived of *M. apelta* aerial parts have not been determined so far, to the best of our knowledge. Only the essential oil of *M. apelta* roots has been determined by GC and GC/MS [8]. Moreover, a literature survey showed that insecticidal activities of the essential oil against grain storage insects were not measured. Thus, the present

investigation consists of two parts: determination of chemical composition of the essential oil of *M. apelta* aerial parts; and evaluation of the essential oil as insecticide/fumigant against two grain storage insect pests, namely, maize weevil (*Sitophilus zeamais* Motsch.) and booklouse (*Liposcelis bostrychophila* Badonnel).

EXPERIMENTAL

Plant material and essential oil extraction

Fresh aerial parts of *M. apelta* (15 kg) were harvested from Lishui City (27.54^{o} N latitude and 119.20 ^{o} E longitude, Zhejiang Province, China) in August 2012. The herb was identified by Dr Liu QR (College of Life Sciences, Beijing Normal University, Beijing 100875, China), and a voucher specimens (ENTCAU-Euphorbiaceae-10012) was deposited at the Department of Entomology, China Agricultural University. The samples was air-dired for two weeks and then ground to powder using a grinding mill (Retsch Muhle, Germany) and was 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 oils were stored in airtight containers in a refrigerator at 4 °C for subsequent experiments.

Insects

S. zeamais was obtained from laboratory cultures maintained in the dark in incubators at 29-30 ^{o}C and 70 - 80 % RH and reared on whole wheat at 12 - 13 % moisture content in glass jars (diameter 85 mm, height 130 mm). Unsexed adult weevils used in all the experiments were about 2 weeks 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).

The booklice (*L. bostrychophila*) were obtained from laboratory cultures in the dark in incubators at 28-30 ^{o}C and 70-80 % RH and reared on a 1: 1:1 mixture, by mass, of milk powder, active yeast, and flour. All the containers housing insects and the Petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene. Laboratory bioassays were done within one week after adult collections.

Gas chromatography-mass spectrometry

The essential oil 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 increased at 10 °C/min to 180 °C held for 1 min, and then increased at 20 °C/min to 280 °C and held for 15 min. The injector temperature was maintained at 270 °C. The sample (1 µl, diluted 100:1 in acetone) was injected, with a split ratio of 1:10. The carrier gas was helium at flow rate of 1.0 ml/min. Spectra were scanned from 20 to 550 m/z at 2 scans/s. Most constituents were identified by gas chromatography by comparison of their Kovats 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 08 and Wiley 275 libraries or with mass spectra from literature [9]. Component relative percentages were calculated based on GC peak areas without using correction factors.

Fumigant toxicity bioassay

Fumigant toxicity of *M. apelta* essential oil against maize weevils was determined by used the method of Liu and Ho [10]. A serial dilution of the essential oil (5.0, 7.0, 9.0, 12.0, 17.0, and 22.0 %) was prepared in n-hexane. A Whatman filter paper (CAT no. 1001020, 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 microliters of an appropriate concentration of the essential oil 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. Preliminary experiments demonstrated that 15 s was sufficient for the evaporation of solvents. The vials were upright and the Fluon® coating restricted the insects to the lower portion of the vial to prevent them from the treated filter paper. *n*-Hexane was used as a control. They were incubated at 27 - 29 ^{o}C and 70 - 80 % RH for 24 h. Five replicates were carried out for all treatments and controls. The insects were

considered dead if appendages did not move when probed with a camel brush.

The fumigant toxicity of the essential oil against *L. bostrychophila* was determined as described by Zhao *et al* [11]. A filter paper strip (3.5 cm × 1.5 cm) treated with 10 µl of an appropriate concentration of the essential oil in acetone. The impregnated filter paper was then placed in the bottom cover of glass bottle of 250 ml. The insects, 10 adults in a small glass bottle (8 ml), were exposed for 24 h and each concentration with five replicates. Six concentrations (0.7, 1.0, 1.5, 2.0, 3.0 and 5.0 %) were used in all treatments and controls. Acetone was used as controls and dichlorvos was used as a positive control. Dichlorvos (99.9 %) was purchased from Aladdin-reagent Company (Shanghai).

Contact toxicity test (topical application)

Serial dilutions of the essential oil (5.0 - 25.0 % v/w) were prepared in n-hexane. Aliquots of 0.5 µl of the dilutions were applied topically to the dorsal thorax of the weevils, using a Burkard Arnold microapplicator. Controls were determined using n-hexane. Both treated and control insects were then transferred to glass vials (10 insects per vial) with culture media and kept in incubators. The mortality of the weevils was observed after 24 h.

Contact toxicity test (treated filter paper)

The essential oil was diluted in acetone. The filter paper with 3.5 cm in diameter (Whatman) was treated with 150 µl of the solution. Then the filter paper after treated with solid glue (Glue Stick, Jong Ie Nara Co Ltd, Hong Kong) was placed in a Petri dish (3.5 cm diameter) and 10 booklice were put on the filter paper by using a hair brush. The plastic cover with holes was put and all the Petri dishes were kept in incubators at 27 - 29 ^{o}C, 70 - 80 % r.h. for 24 h. Acetone was used as controls and pyrethrum extract was used as a positive control. Six concentrations (1.0 - 4.5 %) and five replicates of each concentration were used in all treatments and controls. Pyrethrum extract (25 % pyrethrin I and pyrethrin II) was purchased from Fluka Chemie (Buchs, Switzerland).

Data analysis

The observed mortality data were corrected for control mortality using Abbott's formula [12] and the results subjected to probit analysis using the PriProbit Program V1.6.3 to determine LC_{50} or LD_{50} values [13] at the 0.05 level of significance.

RESULTS

The yellow essential oil yield of *M. apelta* aerial parts was 0.21 % w/v and the density of the concentrated essential oil was determined as 0.89 g/ml. A total of 36 components were identified in the essential oil of *M. apelta* aerial parts, accounting for 97.75 % of the total oil (Table 1). The main compounds found were β-eudesmol (18.65 %), β-caryophyllene (9.83 %), β-selinene (6.55 %), caryophyllene oxide (6.29 %), bornyl acetate (6.07 %), γ-eudesmol (5.40 %) and α-selinene (5.06 %) (Table 1). Sesquiterpenoids represented 19 of the 36 compounds, corresponding to 76.69 % of the whole oil while 14 of the 36 constituents were monoterpenoids (15.74 % of the crude essential oil).

No death of insects was observed in the control under current concentration. The essential oil of *M. apelta* aerial parts possessed contact toxicity against *S. zeamais* adults with an LD_{50} value of 46.69 µg/adult and *L. bostrychophila* with an LD_{50} value of 211.02 µg/cm (Table 2). The essential oil of *M. apelta* also exhibited fumigant toxicity against *S. zeamais* adults and *L. bostrychophila* with LC_{50} values of 48.42 mg/l and 3.21 mg/l, respectively (Table 2).

DISCUSSION

The major compounds in the essential oil of *M. apelta* aerial parts were β-eudesmol, caryophyllene, β-selinene, caryophyllene oxide, bornyl acetate, γ-eudesmol and α-selinene. Chemical composition of the essential oil from aerial parts is quite different from that derived from roots, which contained nerolidol (8.74 %), bis(2-ethylhexyl) adipate (8.08 %), bornylamine (6.79 %), 1,6-octadien-3-ol (5.57 %), and 2,7-dimethyl-1,6-octadiene (5.18 %) [8].

The essential oil exhibited contact toxicity against *S. zeamais* adults and *L. bostrychophila*. However, the essential oil of *M. apelta* demonstrated one-eleventh as acute toxic as the pyrethrum extract against *S. zeamais* (LD_{50} = 4.29 µg/adult) [15] and *L. bostrychophila* (LD_{50} = 18.99 µg/cm) [11]. The essential oil of *M. apelta* also exhibited fumigant toxicity against *S. zeamais* adults and *L. bostrychophila*. The commercial grain fumigant, methyl bromide (MeBr) exhibited fumigant activity against

Table 1: Chemical constituents of essential oil of *Mallotus apelta* aerial parts

Peak no.	Compound	RI*	%
1	α-Pinene	939	0.09
2	β-Pinene	981	0.58
3	β-Myrcene	992	0.32
4	*o*-Cymene	1022	0.17
5	(d)-Limonene	1030	2.48
6	1,8-Cineol	1029	1.84
7	(*Z*)-β-Ocimene	1037	1.21
8	γ-Terpinene	1059	0.19
9	*cis*-Linalool oxide	1067	0.27
10	Linalool	1094	0.89
11	Camphor	1146	0.96
12	2,6-Octadien-1-ol	1232	2.05
13	Geraniol	1254	0.79
14	**Bornyl acetate**	**1286**	**6.07**
15	Eugenol	1356	3.05
16	Methyleugenol	1403	2.69
17	**β-Caryophyllene**	**1420**	**9.83**
18	*trans*-α-Bergamotene	1432	0.92
19	Aromadendrene	1441	0.63
20	Geranyl acetone	1453	0.88
21	α-Caryophyllene	1454	2.25
22	γ-Selinene	1472	3.71
23	**β-Selinene**	**1490**	**6.55**
24	**α-Selinene**	**1494**	**5.06**
25	*cis*-Nerolidol	1535	2.42
26	(-)-Spathulenol	1578	2.22
27	**Caryophyllene oxide**	**1583**	**6.29**
28	Humulene oxide II	1608	0.88
29	**γ-Eudesmol**	**1631**	**5.40**
30	Hinesol	1637	1.03
31	**β-Eudesmol**	**1648**	**18.65**
32	α-Eudesmol	1653	3.47
33	α-Cadinol	1654	1.31
34	Agarospirol	1664	1.91
35	β-Bisabolol	1673	0.47
36	Phytol	2119	0.22
	Total		97.75
	Monoterpenoids		15.74
	Sesquiterpenoids		76.69
	Other		5.32

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

Table 2: Contact and fumigant toxicity of *Mallotus apelta* essential oil against *Sitophilus zeamais* (SZ) adults and *Liposcelis bostrychophila* (LB)

Insect	Treatment	Contact toxicity			Fumigant toxicity		
		LD_{50} (μg/adult) (μg/cm) (%FL)	Slope ± SE	Chi square (χ^2)	LC_{50} (mg/l air) (%FL)	Slope ± SE	Chi square (χ^2)
SZ	*M. apelta*	46.69 (43.95-49.34)	6.99 ± 0.74	13.72	48.42 (45.53-51.24)	6.66 ± 0.71	11.76
	Pyrethrum extract*	4.29 (3.86-4.72)	-	-	-	-	-
	MeBr*	-	-	-	0.67	-	-
LB	*M. apelta*	211.02 (196.34-227.06)	4.99 ± 0.52	10.08	3.21 (2.98-3.46)	4.88 ± 0.51	15.96
	Pyrethrum extract*	18.99 (17.56-20.06)	-	-	-	-	-
	Dichlorvos*	-	-	-	1.35×10^{-3} ($1.25-1.47)\times10^{-3}$	-	-

Sources: *****Li *et al* [14]; Liu and Ho [10]; Zhao *et al* [11]

S. zeamais adults with an LC_{50} value of 0.67 mg/l thus the essential oil was 1/72 as toxic as MeBr to *S. zeamais* adults [10].

Moreover, the essential oil exhibited only 2400 times less toxicity than dichlorvos against the booklice because dichlorvos was reported to exhibit fumigant toxicity against *L. bostrychophila* with an LC_{50} value of 1.35×10^{-3} mg/l [11]. However, compared with other essential oils in the previous studies that were tested using a similar bioassay, the essential oil of *M. apelta* aerial parts exhibited stronger or similar level of fumigant toxicity against *S. zeamais* adults, e.g., the essential oils of *Aster ageratoides* [13], *Murraya exotica* [14], *Ostericum sieboldii* [16] and several essential oils from Genus *Artemisa* [17,18]. Moreover, the main constituent, β-eudesmol, has been demonstrated to possess contact toxicity and ovicidal activity against diamondback moth, *Plutella xylostella* [19], and acute toxicity against the common vinegar fly, *Drosophila melanogaster* was also observed [20]. Thus, the isolation and identification of the bioactive constituent compounds in the essential oil of *M. apelta* aerial parts are of utmost importance so that their potential application in controlling stored-product pests can be fully exploited.

CONCLUSION

Our findings suggest that the fumigant activity of the essential oil of *M. apelta* aerial parts is promising considering the currently used fumigants which are synthetic in origin. Since currently used fumigants are synthetic insecticides and the most effective fumigants are also highly toxic to humans and other non-target organisms, the essential oil of *M. apelta* aerial parts can play an important role in stored grain protection and also reduce the risks associated with synthetic insecticides. However, further studies on the safety of the essential oil in humans and also development of formulations are necessary to improve the efficacy and stability as well as reduce cost.

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