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GC-MS Analysis of insecticidal essential oil of aerial parts of *Artemisia anomala* S. Moore against *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae)

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Abstract
Water-distilled essential oil from the aerial parts at flowering stage of *Artemisia anomala* (Compositae) was analyzed by gas chromatography-mass spectrometry (GC-MS). Thirty-three compounds, accounting for 97.46% of the total oil, were identified and the main components of the essential oil of *A. anomala* aerial parts were camphor (15.47%), 1,8-cineole (15.41%), caryophyllene oxide (9.27%), borneol (7.78%), and caryophyllene (6.55%) followed by spathulenol (4.57%) and carvone (4.32%). The essential oil has higher content of (55.27%) of sesquiterpenoids than monoterpenoids (40.29%). The essential oil of *A. anomala* aerial parts possessed strong fumigant toxicity against the booklice (*Liposcelis bostrychophila*) with an LC_{50} value of 4.05 mg/l. The essential oil also exhibited contact toxicity against *L. bostrychophila* with an LC_{50} value of 131.59 μg/cm². The results indicate that the essential oil of *A. anomala* aerial parts has potential for development into natural insecticides or fumigants for control of insects in stored grains.

Key words: *Artemisia anomala*; *Liposcelis bostrychophila*; Fumigant; Contact Toxicity; Essential Oil

1. 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 [1, 2]. During a screening program for new agrochemicals from Chinese medicinal herbs and local wild plants, the essential oil of *Artemisia anomala* S. Moore (Compositae) aerial parts at flowering stage was found to possess insecticidal activity against the booklice (*Liposcelis bostrychophila* Badonnel). Booklice were regarded as secondary pests, often overlooked due to their small size and the existence of other more damaging post-harvest primary pests e.g. maize weevils (*Sitophilus zeamais*), rice weevils (*S. oryzae*) and lesser grain borer (*Rhyzopertha dominica*) in cereal grains [3, 4]. However, new evidence indicates that psocids are perhaps the most important emerging pests in stored grains and related commodities due to their small size, and resistance to chemicals [4, 5]. Currently, recommended pest control measures in durable stored food products rely heavily on use of synthetic insecticides and fumigants which pose possible health hazards to warm-blooded animals, risk of environmental pollution, development of resistance by insects and pest resurgence [6]. Moreover, populations of *L. bostrychophila* have shown resistance to current used fumigants and contact insecticides [5, 7]. Thus, there is an urgent need to develop safer, environmentally friendlier and efficient alternative that have potential to replace synthetic insecticides and fumigants. In line with this, essential oils or their constituents may provide an alternative to currently used fumigants/pesticides to control stored-food insects [8, 9]. Essential oils of several Chinese *Artemisia* species were demonstrated to possess insecticidal activities to grain storage insects [10-19].

Artemisia anomala is a perennial herbaceous plant (80-150 cm tall) and has been commonly used in traditional Chinese medicines as an analgesic, antibiotic, and as a wound healing agent [20]. It is distributed in eastern and southern China such as Guangdong, Guangxi, Guizhou, Henan, Hubei, Hunan, Anhui, Fujian, Jiangsu, Jiangxi, and Zhejiang Province as well as Taiwan [21]. The aerial parts of this species are usually processed into beverage in some regions in China to relieve summer heat and dyspepsia [22]. The methanol extract of *A. anomala* exhibited antioxidative and antibacterial activity [23]. Previous phytochemical investigations led to the isolation of flavonoids, coumarins, sesquiterpene lactones and prostaglandin-like fatty acid derivatives from *A. anomala* [24-35]. Chemical composition of the essential oil of *A. anomala* has been analyzed previously [36-38]. The essential oil of *A. anomala* possessed antimicrobial and antiproliferative activities against six micro-organisms and four human tumor cell lines [36]. However, a literature survey has shown that there is no report on insecticidal activity of the essential oil of *A. anomala* aerial parts. The present investigation was therefore undertaken to investigate the chemical constituents and insecticidal activities of the essential oil against the booklice.

2. Materials and Methods

2.1. Insects

The booklice (*L. bostrychophila*) were obtained from laboratory cultures in the dark in incubators at 28-30 °C and 70-80% relative humidity (RH) and was 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 (Fluon®, Blades Biological, Edenbridge, UK). Laboratory bioassays were done within one week after adult collections.

2.2. Chinese medicinal herb and essential oil extraction

Fresh aerial parts at flowering stage of *A. anomala* (20 Kg) were harvested from Lishui City, Zhejiang Province, China (27.54° N latitude and 119.20° E longitude) in August 2013. The plant was identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing, China), and a voucher specimen (no. ENTCAU- Compositae-10305) was deposited at the herbarium of Department of Entomology, China Agricultural University. The sample was air-dried and then grinded to powdered form using a grinding mill. It 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. The essential oil was stored in airtight containers in a refrigerator at 4 °C for subsequent experiments.

2.3. GC-MS analysis

Analyses of volatile constituents were determined using an Agilent 5973 GC-MS system operating in the EI mode at 70 eV [equipped with a 30 m HP-5MS column (0.25 mm × 30 m × 0.25 μm) and coated with 5% phenyl-methylpolysiloxane using a HP-5MS (df = 0.25 μm) (Agilent J&W Scientific, USA)]. The temperature program used for the analysis was as follows: initial temperature at 60 °C, held for 1 min, ramped at 4 °C /min to 290 °C and held for 0.5 min. Helium was the carrier gas at 1.0 ml/min; the sample (1 μl, diluted to 1/100, v/v, in hexane) was injected in the split mode (1:5). The injector and detector temperatures were preformed at 230 °C and 300 °C, respectively. The Kovats retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C_8-C_{24}. Quantification was performed using percentage peak area calculations and the identification of individual compartments was done using the Wiley/ NBS Registry of Mass Spectral Database and NIST MS Search, literature [39] and several authentic compounds. The relative concentration of each compound in essential oil was quantified based on the peak area integrated by the analysis program.

2.4. Contact toxicity

Contact toxicity of the essential oil of *A. anomala* against the booklice was measured as described by Zhao *et al.* [40]. Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. The filter paper with 3.5 cm in diameter (Whatman) was treated with 150 μl of the solution (2.0%, 2.4%, 2.9%, 3.5%, 4.2%, and 5.0% in acetone). 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 in diameter) and 10 booklice were put on the filter paper. The plastic cover with holes was put and all the peteri dishes were kept in incubators at 27-29 ^{0}C, 70-80% RH for 24 h and mortality of insects was observed. Acetone was used as controls and pyrethrum extract was used as a positive control. Pyrethrum extract (25% pyrethrin I and pyrethrin II) was purchased from Fluka Chemie (Buchs, Switzerland).

2.5. Fumigant toxicity bioassay

Fumigant toxicity of the essential oil against the booklice was determined as described by Zhao *et al*. [40]. Range-finding studies were run to determine the appropriate testing concentrations of the essential oil. A filter paper strip (3.5 cm×1.5 cm) treated with 10 μl of an appropriate concentration (3.1%, 3.3%, 3.5%, 3.6%, 3.8%, and 4.0% in acetone) of the essential oil/compounds. The impregnated filter paper was then placed in the bottom cover of glass bottle of 250 ml. The insects, 10 adults with undefined sex in a small glass bottle (8 ml), were exposed for 24 h at 27-29 °C and 70-80% relative humidity and each concentration with five replicates. Mortality of insects was observed. Dichlorvos (99.9%) was purchased from Aladdin-reagent Company (Shanghai, China) and used as a positive control.

2.6. Statistical analysis

The observed mortality data were corrected for control mortality using Abbott's formula. The results from all replicates in fumigant and contact toxicity were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LD_{50} and LC_{50} values with their fiducial limits, respectively [41].

3. Results and Discussion

3.1. Chemical composition of the essential oil

The water distillation for 6 h of *A. anomala* aerial parts afforded essential oil (yellow) with a yield of 0.23% (v/w) and the density of the concentrated oil was 0.88 g/ml. Thirty-three compounds were identified and the main components of the essential oil of *A. anomala* aerial parts were camphor (15.47%), 1,8-cineole (15.41%), caryophyllene oxide (9.27%), borneol (7.78%), and caryophyllene (6.55%) followed by spathulenol (4.57%) and carvone (4.32%) (Table 1). The results were quite different from the previous reports [36-38]. For example, the essential oil of *A. anomala* aerial parts collected from Mt. Tianmu located in Zhejiang Province mainly contained camphor (18.3%), 1, 8-cineole (17.3%), β-caryophyllene oxide (12.7%), and borneol (9.5%) [36] while borneol (7.44%), caryophyllene oxide (7.15%), camphor (7.01%) and butylated hydroxytoluene (5.05%) were the major constituents in the essential oil of *A. anomala* aerial parts purchased from Linan City, Zhejiang province [37]. However, the essential oil of *A. anomala* aerial parts from Guiyang city, Guizhou province contained *ar*-curcumene (36.37%), *trans*-farnesene (11.37%), linalool (6.35%), and *trans*-caryophyllene (5.76%) [38]. The above findings suggested there were great geographic variations in chemical composition of the essential oil of *A. anomala* aerial parts. For practical use, it is necessary to standardize the essential oil of *A. anomala* aerial parts.

Table 1: Chemical constituents of the essential oil derived from *Artemisia anomala* aerial parts

Peak no.	Compounds	RI[a]	Composition (%)
1	α-Pinene[b]	931	0.56
2	Camphene	954	0.84
3	Sabinene	975	1.17
4	β-Pinene[b]	981	1.26
5	3-Octanol	993	0.92
6	δ-2-Carene	1002	0.49
7	(d)-Limonene[b]	1030	0.25
8	1,8-Cineole[b]	1032	15.41
9	*cis*-β-Ocimene	1037	0.19
10	γ-Terpinene	1059	0.34
11	Linolool[b]	1094	0.59
12	Camphor[b]	1146	15.47
13	Borneol[b]	1175	7.78
14	4-Terpineol[b]	1177	0.23
15	α-Terpineol[b]	1188	0.67
16	Carvone[b]	1254	4.32
17	Bornyl acetate	1287	2.45
18	Carvacrol	1317	2.75
19	Copaene	1374	3.45
20	Isoledene	1377	1.75
21	Caryophyllene[b]	1420	6.55
22	*trans*-β-Farnesene	1452	1.67
23	α-Caryophyllene	1454	2.88
24	Germacrene D	1458	1.33
25	Viridflorene	1497	1.13
26	Germacrene B	1561	0.81
27	Spathulenol	1578	4.57
28	Caryophyllene oxide[b]	1583	9.27
29	Viridiflorol	1588	0.87
30	Humulene oxide II	1606	3.26
31	τ-Cadinol	1642	0.65
32	β-Eudesmol	1645	1.53
33	β-Bisabolol	1683	0.57
	Total		97.46
	Monoterpenoids	17/33	55.27
	Sesquiterpenoids	15/33	40.29
	Others	1/33	0.92

[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.

3.2. Insecticidal activities of the essential oil

The essential oil of *A. anomala* aerial parts possessed contact toxicity against the booklice with an LC_{50} value of 131.59 μg/cm^2 (Table 2). Compared with the positive control, pyrethrum extract (LC_{50} = 18.99 μg/cm^2), the essential oil exhibited 7 times less active against the booklice. However, compared with the other essential oils in the previous studies using the same bioassay, the essential oil of *A. anomala* exhibited stronger acute toxicity against the booklice, e.g. essential oils of *A. rupestris* (LC_{50} = 414.48 μg/cm^2) and *A. frigida* (LC_{50} = 254.38 μg/ cm^2) [18, 19], *Curcuma wenyujin* (LC_{50} = 208.85 μg/cm^2) [42] and *Valeriana jatamansi* (LC_{50} = 236.4 μg/cm^2) [43]. But less than the essential oils of *Ageratum houstonianum* (LC_{50} = 64.0 μg/cm^2) [44], *Acorus calamus* (LC_{50} = 100.21 μg/cm^2) [45], and *Foeniculum vulgare* (LC_{50} = 90.36 μg/cm^2) [40].

The essential oil of *A. anomala* aerial parts also exhibited fumigant toxicity against the booklice with an LC_{50} value of 4.05 mg/l (Table 2). Compared with the positive control, dichlorvos (LC_{50} =1.35 μg/l), the essential oil was 3000 times less toxic against the booklice. However, the essential oil of *A. anomala* possessed stronger fumigant toxicity against the booklice than essential oils of *A. rupestris* (LC_{50} = 6.67 mg/l) [18], *V. jatamansi* (LC_{50} = 5.98 mg/l) [43] and *A. houstonianum* (LC_{50} > 50 mg/l) [44]. As currently used fumigants are synthetic insecticides and the most effective fumigants are also highly toxic to humans and other non-target organisms, fumigant activity of the essential oil of *A. anomala* aerial parts is quite promising and the essential oil of *A. anomala* aerial parts shows potential to be developed as a possible natural fumigants/insecticide for the control of the booklice.

The two major constituents of the essential oil, camphor and 1,8-cineole had been demonstrated to exhibit contact toxicity against the booklice with LC_{50} values of 207.26 μg/cm^2 and 1048.75 μg/cm^2, respectively while they had fumigant LC_{50} values of 1.03 mg/l air and 1.13 mg/l air, respectively [42]. There is no information on insecticidal activities of the other major constituents of the essential oil against the booklice. The aerial parts of *A. anomala* have been commonly used in traditional Chinese medicines as an analgesic, antibiotic, and as a wounds healing agent [20] and seem to be safe to human consumption. However, for the practical application of the essential oil of *A. anomala* as novel insecticide/fumigant, further studies on the safety of the essential oil to humans and plants are needed. A further study is also necessary to determine the toxicity of the essential oil on other economically important grain storage pests e.g. *Sitophilus*, *Tribolium*, *Callosobruchus* and *Rhyzopertha*. Further studies on the development of formulations are also necessary to improve the efficacy and stability and to reduce cost.

4. Conclusions

The essential oil of *A. anomala* aerial parts demonstrated some contact and fumigant toxicity against the booklice. They showed potential to be developed as possible natural insecticide and fumigant for control of the booklice but needs to be further evaluated for safety in humans and to enhance its activity.

Table 2: Contact toxicity and fumigant toxicity of the essential oil of *Artemisia anomala* aerial parts against *Liposcelis bostrychophila*.

Activity	Treatment	LC_{50}	95% fiducial limits	Slope ± SE	Chi square (χ^2)
Contact ($\mu g/cm^2$)	*A. anomala*	131.59	119.73 - 144.31	7.23 ± 0.65	8.23
	Pyrethrum extract	18.99	17.56 - 20.06	7.64 ± 0.65	-
Fumigant (mg/L air)	*A. anomala*	4.05	3.69 - 4.39	8.18 ± 0.73	7.38
	Dichlorvos	1.35×10^{-3}	$(1.25 - 1.47) \times 10^{-3}$	6.87 ± 0.77	-

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