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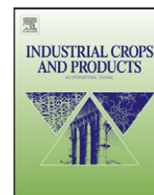

Short communication

Biological activity of essential oils of *Athamanta haynaldii* and *Myristica fragrans* to gypsy moth larvae

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ABSTRACT

Ethanol solutions of essential oils obtained from *Athmanta haynaldii* and *Myristica fragrans* were tested for their toxicity and antifeedant activity against the second instar gypsy moth larvae in the laboratory bioassay. Both isolated oils were subjected to gas chromatography analysis in order to determine their chemical constitutions. Tested oils showed low to moderate larvicidal effect in both residual toxicity test and in chronic larval mortality bioassay. However, antifeedant index achieved by application of tested solutions in feeding choice assay was significantly higher in comparison to control, and almost same as one provided with botanical standard. Low toxic and high antifeedant properties (AF index 85–90%) make these essential oils suitable for integrated pest management programs. Special attention should be paid to further investigation of endemic and rare *A. haynalidii* in the terms its cultivation and usage of its unique set of biologically active compounds.

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1. Introduction

The caterpillars of gypsy moth are major defoliators of deciduous forests in northern hemisphere (Elkinton and Liebhold, 1990). In order to reduce its population density, conventional pesticides were applied frequently in forest management, and biological control measures have been partially introduced in the form of *Bacillus thuringiensis* (Bt) insecticides. However, modern environmental requirements impose the need for expanding the biological control measures. Investigations of biological activity of plant derivatives lead to this goal, and some researchers have demonstrated certain promising natural substances that can be used for this purpose (Marković et al., 1996; Zabel et al., 2002; Kostić et al., 2008). Natural semiochemicals with low-toxic potential which would not cause ecosystem disturbance due to high mortality of target insect population could became the predominant method of pest control in the future (Schumutterer, 1985; Isman, 2006). Essential oil *Myristica fragrans* has been widely exploited and showed the number of biological activities against pest insects (Suryakala et al., 2007). On the other hand, endemic and rare species *Athamanta haynaldii* has not been considered as the potential natural resource, and there is no data about biological activity of its essential oil. Given the similar chemical composition of two plants and extremely high content of myristicin in essential oil of *A. haynaldii*, we hypothesized that certain biological activities will be demonstrated in biotest.

2. Materials and methods

2.1. Plant material

A. haynaldii Borb. et Uecht. (Apiaceae) plants were collected from locality Ovčarsko Kablarska Gorge, Serbia during the balmy period; whole plants were air-dried at room temperature (22–25 °C) for 7 days, and used for obtaining of essential oil in a Clevenger-type apparatus (European Directorate for the Quality of Medicines, 2002). Essential oil of *M. fragrans* Houtt. (Myristicaceae) was commercial preparation (Fluka, Buchs, Switzerland). Prior to bioassay, crude oils were diluted with 96% ethanol to prepare test solutions of 0.10% and 0.50%.

2.2. Chemical characterization of obtained fractions

The composition of the essential oils was determined by gas chromatography (GC) and mass spectra (MS) analyses, as described

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by Block et al. (2006). GC analyses were performed using HP-5890 Series II gas chromatograph, with split/splitless injector, fused silica capillary column (25 m × 0.32 mm) coated with non-polar stationary phase HP-1 (cross-linked methylsilicone, 0.5 μm film thickness) and flame ionization detector (FID). GC/MS analyses were done on a Hewlett-Packard 5890 gas chromatograph directly coupled to a Hewlett-Packard HP 5971 A (70 eV) mass selective detector. Component identification was carried out by comparing the obtained MS data with those reported in Library Wiley on MS-Chem-Station HP v. B.00.01.

2.3. Botanical insecticide standard

'Bioneem' (0.09% azadirachtin, Safer) was used as botanical standard control (BS); the preparation was diluted with 96% ethanol to prepare test solutions of 0.10% and 0.50%.

2.4. Gypsy moth culture

Gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), egg masses were collected in Opovo (plantation of *Populus × euroamericana*). Eggs were mechanically cleaned from hairs and disinfected (dipped in 0.1% Na-hypochloride for 5 min); than washed with distilled water for 10 min and air dried. Vital eggs from 25 egg masses were intermixed and put into flasks for hatching (at 25 °C). Newly hatched larvae were selected and maintained together in Petri dishes (120 × 15 mm) on the artificial diet (MP Biomedicals, Inc.). They were maintained, and all experiments were carried out, in a microclimate chamber, at 25 ± 1 °C, 65 ± 5% RH and neon diffuse light with 30159.29 candelas with 15:9 h L/D (Odel et al., 1985).

2.5. Contact (residual) toxicity of essential oils

Tested solutions were deposited on the bottom of Petri dishes (R = 9 cm) in the quantity of 0.3 ml, dried about 20 min at 21 °C and than 10 larvae per replication were introduced. Experiments with the BS and control were performed at the same conditions. Dead larvae were removed after 24 and 48 h. To determine whether the larva is alive or dead we used 'palpation method' (touch the larva with soft painting brush – if it make a move it considered alive, otherwise it considered dead). The treatments were replicated six times (n = 60). Percentage insect mortalities were calculated using corrected Abbott formula (Abbott, 1925): Corrected % = $(1 - (T/C)) \times 100$, where T and C were the number of living larvae at treatment and control, respectively, after the observation time.

2.6. Antifeedant activity of essential oils

For this investigation small branches of *Prunus cerasifolia* L. (20 cm long with uniform leaf mass) were used (for gypsy moth, this species is one of the preferably hosts in orchards). Branches were put into flasks with water and then fixed into the pots with sand. Leaf mass was treated by spraying with each tested solution (with TLC sprayer where the solution deposit was 3.0 ± 0.3 mg/cm^2, e.g. a total of 40 ml of solution per m^2 was used for the treatments). When the deposit dried (about 20 min), glass cylinders were put on for the isolation of the treatment and larvae. Then, 10 larvae per replication were introduced. Experiments with both BS and control (ethanol 96%) were performed under the same conditions. Leaf mass damage was evaluated after 24, 48, 72, 96 and 120 h. The treatments were replicated six times (total, n = 60).

2.7. Digestive toxicity of essential oils

Digestive toxicity of essential oils was evaluated following the same experimental design, where we counted dead larvae in the same time intervals. Mortality was expressed in percentages.

2.8. Statistical analysis

Data analysis included the calculation of the mean values and the analysis of variance, where different concentrations of tested essential oils, alcohol and control (untreated) were independent variables. For data on leaf mass damage, analyses of variance were performed with arcsine transformed data. Antifeedant index AF was calculated according to formula: $AF = ((Co - T)/(Co + T)) \times 100$, where Co is for control, T for treatment, and expressed in percentages. Untransformed data from mortality test were used for analysis of variance. Differences between mean values in each set of data were additionally tested by Duncan's multiple range test at 5% level.

3. Results

Chemical analysis showed that essential oil of *A. haynaldii* is composed of 14 components; in essential oil of *M. fragrans* 24 components are detected (Table 1). These oils have 7 common components; the dominant component of *A. haynaldii* essential oil is myristicin (53.48%), and the α-pinen is the dominant component of essential oil of *M. fragrans* (25.07%).

Multivariate analysis of contact (residual) larval mortality showed significant differences among tested essential oils, botanical standard and control (F = 3.78; d.f. = 14, 78; p = 0.0003). The highest mortality was observed in larval group exposed to 0.1% *A. haynaldii* essential oil. Multivariate analysis of digestive mortality and mortality caused with larval starving (over 24–120 h) showed significant differences among tested essential oils, botanical standard and control (F = 13.63; d.f. = 35, 153.9; p < 0.0001). The highest mortality was observed at the 0.1 concentrated essential oils, with the regular time dependent dynamics (mortality increased over time). Leaf mass damage differed significantly according to applied solution (F = 9.29; d.f. = 35, 153.9; p < 0.0001). At the end of experiment, leaf mass damage on the leaves treated with tested essential oils was similar as on those treated with BS, but much lesser than on control leaves (Table 2). Antifeedant index achieved by application of tested solutions was similar as the one achieved with BS and significantly higher than antifeedant index on control leaves (Table 2).

4. Discussion

In terms of integrative pest control and environmental protection, secondary plant metabolites possessing insecticidal, repellent and/or antifeedant properties are very desirable as a possible means of plant protection. According to Schumutterer (1985), essential oils should not adversely affect non-target organisms or environment because of their low toxicity; in preparations, they are present in low concentrations; and, preparations are used in small amounts.

Both tested oils caused a significant decrease of larval feeding in bioassay; their antifeedant index was significantly greater than their toxicity. It could be concluded that volatiles from essential oils inhibited the responses of larvae to specific chemical stimuli that are crucial for host location (Chapman, 1974; Pare and Tumlinson, 1996; Milanović, 2010). Similarly as botanical standard Bioneem, which provide 'primary' (or gustatory) antifeedance (Mordue and Nisbet, 2000), essential oils from *A. haynaldii* and *M. fragrans* showed high potential to mask the host and reduce leaf mass

Table 1
Chemical composition of essential oils of *A. haynaldii* and *M. fragrans* (%m/m).

	RRT[a]	RI[b]	Component	*A. haynaldii*		*M. fragrans*	
				CI[c]	%m/m	I[c]	%m/m
1.	0.256	938	α-Thujone			1	1.29
2.	0.264	942	α-Pinene	8	0.41	000	25.07
3.	0.277	954	Camphene			8	0.46
4.	0.308	976	Sabinene			47	18.73
5.	0.311	981	β-Pinene	144	7.70	50	18.79
6.	0.333	986	β-Myrcene	9	0.47	5	2.14
7.	0.347	1002	α-Phelandrene			7	0.67
8.	0.357	–	δ^3-Carene			2	1.55
9.	0.363	–	α-Terpinene			6	1.65
10.	0.367	1020	p-Cymene			5	1.13
11.	0.377	1025	β-Phelandrene			8	1.95
12.	0.380	1030	Limonene			81	4.54
13.	0.419	1057	γ-Terpinene	3	0.15	08	2.72
14.	0.459	1077	α-Terpinolene			8	1.21
15.	0.471	1092	Linalool			8	2.20
16.	0.572	1175	Terpinene-4-ol			38	3.45
17.	0.588	1185	α-Terpineol			0	0.50
18.	0.711	1278	Safrole			5	1.39
19.	0.800	1350	Eugenole			5	1.14
20.	0.849	–	Metileugenole			5	0.12
21.	0.856	1398	α-Copaene	12	0.66	4	0.60
22.	0.863	1406	β-Burbonene	10	0.56		
23.	0.872	–	$C_{15}H_{24}$ (M = 204)	15	0.78		
24.	0.906	1428	Trans-caryophyllene	70	3.77	0	0.25
25.	0.922	–	$C_{15}H_{24}$ (M = 204)	17	0.89		
26.	0.945	1437	α-Humulene	45	2.40		
27.	0.978	–	Epi-bicyklosesquiphelandrene	378	20.20		
28.	1.000	–	Myristicin	1000	53.48	04	5.12
29.	1.027	–	γ-Elemene	27	1.47		
30.	1.042	–	Elemicin			3	0.33
31.	1.064	1510	δ-Cadinene	68	3.66		

[a] Relative retention time (myristicin = 1.000).
[b] Kovač's retention idex.
[c] Concentration index.

Table 2
Leaf mass damage (mean ± st. error) and antifeedant index (%) of different treatments on the second instar larvae of *L. dispar*.

Variants	C	Leaf mass damage (LMD) and antifeedant index (AF) after									
		24 h		48 h		72 h		96 h		120 h	
		LMD	AF	LMD	AF	LMD	AF	LMD	AF	LMD	AF
A. haynaldii	0.05	1.0 ± 0.00bc	45.45	1.0 ± 0.00c	88.12	1.2 ± 0.17c	92.09	2.8 ± 0.54bc	84.33	3.5 ± 0.50bc	85.59
A. haynaldii	0.1	1.0 ± 0.00bc	45.45	1.0 ± 0.00c	88.12	1.0 ± 0.00c	93.18	1.0 ± 0.00c	94.17	2.0 ± 0.00c	90.28
M. fragrans	0.05	0.7 ± 0.21bc	60.00	0.7 ± 0.21c	91.92	0.8 ± 0.31c	94.29	1.3 ± 0.33c	92.31	2.0 ± 0.00c	90.28
M. fragrans	0.1	1.0 ± 0.00bc	45.45	1.5 ± 0.34c	82.69	2.3 ± 0.42c	84.78	2.7 ± 0.56bc	85.19	3.2 ± 0.40bc	85.04
Alcohol	96	2.2 ± 0.40a	10.34	11.2 ± 0.83b	17.28	24.2 ± 1.54b	7.94	31.7 ± 2.47a	2.56	35.8 ± 2.39a	4.44
Bioneem	0.05	1.3 ± 0.33b	33.33	3.0 ± 1.10c	68.14	3.5 ± 1.44c	78.01	5.8 ± 2.04b	70.21	7.2 ± 2.02b	69.06
Bioneem	0.1	0.2 ± 0.17c	88.24	1.3 ± 0.76c	84.47	2.2 ± 0.79c	85.49	2.8 ± 0.91bc	84.76	3.3 ± 0.92bc	84.31
Control	0.0	2.7 ± 0.56a	0.0	15.8 ± 1.54a	0.0	28.3 ± 1.050a	0.0	33.3 ± 1.05a	0.0	39.2 ± 1.54a	0.0

C, concentration. Different letters marks significant differences (Duncan test, $p < 0.05$).

damage caused by larval feeding. Chemical analysis showed that one of the constituents of both oils is highly biologically active myristicin. Besides, several volatile monoterpens and sesquiterpens with strong biological activity have been detected (α-pinene, β-pinene, sabinene, epi-bicyclosesquipelantrene). Synergistic effects of complex mixtures, as essential oils, are thought to be important in plant defenses against herbivory. Plants usually present defenses as a set of compounds, achieving the effect of the dominant component through various mechanisms. Thus, complex essential oils are significantly more efficient than the pure compounds derived from them (Don-Pedro, 1996; Hori, 1998). Recent reports indicate a strong antifeeding effect of plant derivatives and recommend their widespread use because of pronounced environmental safety and lack of insect desensitization (Xu et al., 2009; Khosravi et al., 2010; Sandoval-Mojica and Capinera, 2011; Akhtar et al., 2012). Presented results prove that the application of natural plant products possessing highly biologically active compounds in terms of antifeedant and/or masking effect and low toxicity on *L. dispar*, can be potential method in integrative control management of this pest. Also, these results are indicative for further investigation on so far under-researched biological activities of *A. haynaldii*.

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