

Chemical Composition and Activity of Essential Oils of Albanian Coniferous Plants on Plant Pests [†]

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Abstract: The present study was conducted to evaluate and compare the chemical composition and bioactivity of essential oils on different insect organisms from four Albanian coniferous plants. The phytochemical analysis carried out using GC-MS showed that the oils were constituted mainly by monoterpenes, sesquiterpenes and diterpenes. Chemical analysis identified 16 constituents in black pine and silver fir, and 17 constituents in Douglas fir, whereas the analysis allowed identification of 28 constituents in juniper berry. Main constituents of the essential oils included α -pinene and *c*-verbenol in black pine; β -pinene, α -fenchone and α -pinene in Douglas fir; limonene, β -pinene, α -pinene and camphene in silver fir; and α -pinene, sabinene and β -myrcene in juniper berry. The oils showed varying degrees of insecticidal activity. Juniper berry and silver fir affected the settling behavior of the aphids *Myzus persicae* and *Rhopalosiphum padi*, respectively. Black pine oil presented significant activity against the tick *Hyalomma lusitanicum* and the root-knot nematode *Meloidogyne javanica*. The ixodicidal effect of this essential oil was explained by its content in *c*-verbenol, whereas the compound and binary combinations of α -pinene and *c*-verbenol were not nematocidal, suggesting synergic effects between minor components of black pine essential oil.

Keywords: coniferous plant; essential oil; insecticidal; ixodicidal; nematocidal



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1. Introduction

Insect control in conventional agriculture systems is conducted primarily by the use of synthetic pesticides. However, their inappropriate use has led to environmental problems such as soil and water pollution, toxicity to predators and pollinators (beneficial insects) and, in some cases, they are found as residues in food products. Nowadays, there is an increased demand for alternative biodegradable pesticides with low impact on the environment. Because of their high volatility, essential oils (EOs) or their constituents are considered a potential substitute (partially or completely) to synthetic insecticides [1]. EOs from woody coniferous plants are rich in monoterpenes, sesquiterpenes and diterpenes [2,3]. Compounds belonging to these chemical classes have shown different biological activities. A few data are available regarding chemical composition of essential oils from Albanian conifers. The present investigation aims to study chemical constituents of coniferous essential oils and their possible insecticidal, acaricidal and nematocidal activity.

2. Materials and Methods

EOs of silver fir (*Abies alba*), juniper berry (*Juniperus communis*), Douglas fir (*Pseudotsuga menziesii*), and black pine (*Pinus nigra*) of wild origin were provided from the Albanian company “Mediterranean Spices & Imports”, Tiranë. Young shoots of *A. alba*, *P. menziesii* and

P. nigra collected in August–October were steam distilled for four hours, whereas berries of *J. communis* collected in August–November were steam distilled for 36 h in intervals.

2.1. Chemical Analysis of Essential Oils

The extracted EOs were analyzed by gas chromatography–mass spectrometry (GC-MS) as described by Mamoci et al. [4]. Electron ionization mass spectra and retention data were used to assess the identity of compounds by comparing them with standards or those found in the Wiley 229 Mass Spectral Database.

2.2. Insect and Acaricidal Bioassays

Spodoptera littoralis was reared on an artificial diet, whereas *M. persicae* and *R. padi* were cultivated on their host plants (*Capsicum annuum* var. California Wonder s/calibra, Ramiro Arnedo S.A, Calahorra, Spain and *Hordeum vulgare*, La Poveda-ICA/CSIC, Arganda Del Rey) and kept in a growth chamber at 22 ± 1 °C, >70% relative humidity (RH) with a photoperiod of 16:8 h (L:D). The bioassays were carried out with newly emerged *S. littoralis* L6 larvae or ten *M. persicae*/*R. padi* adults as described by Burgueño-Tapia et al. [5].

Spanish populations of *Hyalomma lusitanicum* from Central Spain (Ciudad Real and Madrid) were used. Engorged female ticks were collected on hosts (deer) and maintained at 22–24 °C and 70% RH until oviposition. The eggs were kept under the same environmental setting until they hatched. Four- to six-week-old larvae were used for the bioassays carried out as described by González-Coloma et al. [6].

2.3. Nematicidal Bioassay

A *Meloydogine javanica* population, maintained on *Solanum lycopersicum* plants (var. Marmande) in pot cultures at 25 ± 1 °C at >70% relative humidity, was used. Second-stage juveniles (J2) hatched within a 24 h period from egg masses and handpicked from infected tomato roots were used in this bioassay. The experiments were carried out in 96-well microplates (Becton, Dickinson) as described by Andrés et al. [7]. The EOs and pure compounds were tested at initial concentrations of 1.0 and 0.5 mg/mL, respectively (final concentration in the well). The number of dead juveniles was recorded after 72 h. All treatments were replicated four times. The data were determined as percent mortality corrected according to Schneider-Orelli's formula.

3. Results and Discussion

3.1. Chemical Analysis of Essential Oils

In Table 1, phytochemical constituents of essential oils using GC-MS are presented.

Table 1. Main constituents of the studied essential oils.

Ret. Time	Base m/z	Compound	% Area			
			Black Pine	Douglas Fir	Silver Fir	Juniper Berry
4.95	93.10	α -Thujene/ α -phellandrene		1.46		2.29
5.12	93.10	α -Pinene	41.99	10.89	13.33	24.34
5.44	93.10	Camphene	5.25	1.47	11.13	0.35
5.95	93.10	Sabinene		8.45		13.86
6.07	93.10	β -Pinene	1.13	24.78	18.39	3.14
6.33	93.10	β -Myrcene		1.60	0.83	11.46
6.85	93.10	δ -3-Carene		4.54		
7.02	121.15	α -Terpinene		3.49		1.39
7.26	119.10	<i>p</i> -Cymene	2.94	2.37	0.47	2.19
7.35	93.10	Sylvestrene		4.15		

Table 1. Cont.

Ret. Time	Base <i>m/z</i>	Compound	% Area			
			Black Pine	Douglas Fir	Silver Fir	Juniper Berry
7.38	68.05	Limonene	3.81		20.24	5.75
7.45	43.00	1,8-Cineole			9.06	
8.19	93.10	γ -Terpinene		5.90		2.74
9.13	81.05	α -Fenchone	0.50	12.93		
9.15	93.1	Terpinolene			0.39	1.89
9.47	71.05	Linalool			2.13	
9.68	43.00	43/109/67/69/82/95/91/41/79/94	7.72			
10.29	108.1	alpha-Campholene aldehyde	5.60			
10.72	92.05	Trans-pinocarveol	3.68			
10.91	109.1	<i>c</i> -Verbenol	11.26			
11.61	95.1	Borneol	0.70		0.34	
11.94	71.05	4-Terpineol		10.84		4.43
12.46	59.05	l- α -Terpineol	0.99	1.62	0.86	
12.63	79.05	Myrtenol	2.19			
13.05	107.1	Berbenone	4.33			
13.41	109.1	Trans-carveol	0.93			
15.60	95.1	Endobornyl acetate		0.83	9.94	0.38
17.00	91.05	Myrtenyl acetate			6.54	
17.83	121.15	α -Terpinenyl acetate			0.57	
17.85	105.1	α -Cubebene				0.74
17.86	81.10	Citronellyl acetate		3.58		
18.89	69.10	Neryl acetate		0.58		
19.26	93.1	β -Elemene				2.31
20.18	93.1	β -Trans-caryophyllene			0.89	3.16
20.61	121.1	γ -Elemene				4.03
21.28	93.1	α -Humulene			0.70	2.33
22.01	161.15	α -Amorphene	0.71			0.92
22.16	161.15	Germacrene-D				4.30
22.33	105.1	β -Selinene				0.52
22.62	107.1	α -Selinene				0.81
22.77	105.1	α -Muurolene(-)				0.91
23.20	161.15	Germacrene-D isomer				1.06
23.48	161.15	δ -Cadinene				3.17
23.94	105.1	Aristolene				0.74
24.54	161.15	Germacrene-D				0.44

3.2. Insecticidal and Acaricidal Activity

Table 2 shows the antifeedant effects of the essential oils against three different species of phytophagous insects. Among these insects, only the aphids were affected by the EOs. Silver fir EO showed the highest antifeedant activity against *M. persicae*, whereas juniper berry EO was the strongest antifeedant against *R. padi*. The major components of these oils (limonene and α -pinene) were not active against *M. persicae* or *R. padi*, suggesting additive or synergistic effects of other minor components. None of these EOs affected the polyphagous lepidopteran *S. littoralis*. The essential oil of *J. communis* (at 10%) gave a 100% mortality of the aphid *Aulacorthum solani* Kalt. [8]. The essential oil of Douglas fir showed a 100% mortality through direct spraying against the aphid *Phyllaphis fagi* at a concentration of 1% [9].

Table 2. Insect antifeedant activity of coniferous essential oils.

Essential Oils	$\mu\text{g}/\text{cm}^2$	<i>M. persicae</i> SI%	<i>R. padi</i> SI%	<i>S. littoralis</i> FI%
Black pine	100	69.8 ± 8.1	47.4 ± 7.7	30.6 ± 6.6
Douglas fir	100	48.2 ± 9.2	69.4 ± 6.4	55.5 ± 6.2
Juniper berry	100	66.9 ± 9	79.7 ± 5.2	26.4 ± 12.5
	50		68.1 ± 6.7	
	25		35.4 ± 8.4	
Silver fir	100	76.5 ± 6.7	54.5 ± 7.1	19.2 ± 5.7
	50	38.1 ± 9.4		
	25	21.1 ± 6.5		
Limonene	50	29.3 ± 7.7		
α -Pinene	50	53.9 ± 10.2	34.8 ± 8.1	

SI% (Settling inhibition index) = $1 - (\%T/\%C) \times 100$, where %T and %C are the percentages of aphids on treatment and control surface, respectively. FI%, percent feeding inhibition \pm standard error.

When tested against the tick *Hyalomma lusitanicum*, black pine EO showed a strong dose-dependent larvicidal effect. This larvicidal effect can be explained by the EO content in c-verbenol as shown in Table 3. *H. lusitanicum* is a tick responsible for the transmission of the blood parasite *Theileria annulata* (protozoon) that causes Mediterranean theileriosis in cattle [10] and their bites may cause bacterial infections [11]. To our knowledge, this is the first report that shows the acaricidal activity of black pine essential oil on *H. lusitanicum*. Monoterpene verbenol was reported to have anti-ischemic and anti-inflammatory activity [12]. This compound was also reported as a repellent of the *Ixodes ricinus* (L.) tick [13].

Table 3. Mortality of *Hyalomma lusitanicum* treated with four essential oils and the major components of black pine.

$\mu\text{g}/\text{mg}$ Cellulose	Mortality (%) ^a					
	Douglas Fir	Silver Fir	Juniper Berry	Black Pine	α -Pinene	c-Verbenol
2.0	8.9 ± 7.5	30.41 ± 13	0.0 ± 0	100 ± 0		
1.0				94.6 ± 1.5	1.6 ± 1.6	100 ± 0
0.5				54.2 ± 12.6		100 ± 0
0.25				13.3 ± 4.1		16.9 ± 1.1

^a Corrected according to Schneider-Orelli's formula. Values in the table represent the mean value of three replicates (\pm standard error).

3.3. Nematicidal Activity

The EOs were also tested against the root-knot nematode *Meloidogyne javanica* (Table 4). Our results showed that black pine EO had significant nematicidal effects. The essential oil of stone pine (*Pinus pinea*) exhibited toxic effects against J2 of *M. incognita* [14]. However, this is the first report on the nematicidal activity of black pine essential oil. Neither α -pinene, c-verbenol or their combinations showed nematicidal activity, suggesting synergistic effects for the minor components of the black pine essential oil.

Table 4. Effects of coniferous essential oils on mortality of second stage juveniles (J2) of *M. javanica*.

Treatment	<i>M. javanica</i> J2 Mortality (%) ^a	
	1 µg/mL	0.5 µg/mL
Douglas fir	4.13 ± 0.45	
Silver fir	1.71 ± 0.46	
Juniper berry	3.99 ± 1	
Black pine	81.48 ± 2.42	
α-Pinene		1.82 ± 1.0
c-Verbenol		4.42 ± 0.47
α-Pinene: c-verbenol (50:50)		1.32 ± 0.62
α-Pinene: c-verbenol (90:10)		1.78 ± 0.50

^a Corrected according to Schneider-Orelli's formula. Values are means (±standard error) of four replicates.

4. Conclusions

The major constituent of black pine and juniper berry oil was α-pinene, whereas limonene and β-pinene were the main constituents of silver fir and Douglas fir oil, respectively. Four commercial essential oils from coniferous plants in this study demonstrated under experimental conditions varying degrees of insecticidal activity. This is the first report that shows the bioactivity of the black pine essential oil on the tick *H. lusitanicum* and the nematode *M. javanica*. The acaricidal activity of the black pine oil may be attributed to c-verbenol, whereas the nematocidal activity seems to be due to synergistic interaction of minor compounds rather than from the activity of the main compounds.

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