chemistry proceedings

Proceeding Paper

Chemical composition and activity of essential oils of Albanian coniferous plants on plant pests †

ErjonMamoci[1,*], Maria Fe Andrés[2], Sonia Olmeda[3] and Azucena González-Coloma[2]

[1] Department of Food Science and Biotechnology, Agricultural University of Tirana, KoderKamez, 1029 Tirana, Albania
[2] Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, Serrano 115-bis, 28006 Madrid, Spain; mafay@ica.csic.es (M.F.A.);azu@ica.csic.es (A.G.-C.)
[3] Dpto. Sanidad Animal, Universidad Complutense de Madrid, Avda. Puerta de Hierro, s/n. Ciudad Universitaria, 28040 Madrid,Spain;angeles@ucm.es
* Correspondence: mamocie@ubt.edu.al

† Presented at the 1st International Online Conference on Agriculture—Advances in Agricultural Science and Technology, 10–25 February 2020; Available online: https://iocag2022.sciforum.net/.

Abstract: The present study was conducted to evaluate and compare the chemical composition and bio activity on different insect organisms of essential oils 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, 17 constituents in Dauglas fir, while the analysis allowed identification of 28 constituents in Juniperus berry. Main costituents of the essential oils were 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; α-pinene, sabinene, β-myrcene in Juniperus berry. The oils showed varying degree of insecticidal activity. Juniperus 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, while this compound and binary combinations of α-pinene and *c*-verbenol were not nematicidal, suggesting synergic effects between minor components of Black pine essential oil.

Keywords: Coniferus plant; Essential oil; insecticidal; ixodicidal; nematicidal

Citation: Mamoci, E.; Andrés, M.F.; Olmeda, S.; González-Coloma, A. Chemical composition and activity of essential oils of Albanian coniferous plants on plant pests. *Proceeding Paper* **2022**, *4*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: date

1. Introduction

Insect control in conventional agriculture systems is done 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 in 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 nematicidal activity.

2. Materials and Methods

EOs of Silver fir (*Abies alba*), Juniperus berry (*Juniperus communis*), Douglas fir (*Pseudotsuga menziesii*), and Black pine (*Pinus nigra*) of wild origin were provided from Albanian company "Mediterranean Spices & Imports" Tiranë. *A. alba*, *P. menziesii*, and *P. nigra* young shoots collected in August-October were steam distilled for four hours while berries of *J. communis* collected in August-November was steam distilled for 36 h with intervals.

2.1. Chemical Analysis of Essential Oils

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

2.2. Insect and Acaricidal bioassays

Spodoptera littoralis was reared on artificial diet while *M. persicae* and *R. padi* were cultivated on their host plants (*Capsicum annuum* Var. California worder s/calibra, Ramiro Arnedo S.A, Calahorra, España 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 carry 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 weeks 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, 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 Scheider-Orelli's formula.

3. Results and Discussion

3.1. Chemical Analysis of Essential Oils

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

Table 1. Main constituents of the studied essential oils.

Ret. Time	Base *m*/*z*	Compound	% Area			
			Black Pine	Douglas Fir	Silver Fir	Juniperus 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	*p*-Cymene	2.94	2.37	0.47	2.19
7.35	93.10	Sylvestrene		4.15		
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	*c*-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	Aristolen				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 essential EOs. Silver fir EO showed the highest antifeedant activity against *M. persicae*, while Juniperus 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	μg/cm²	*M. persicae* SI%	*R. padi* SI%	*S. littoralis* 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
Juniperus 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) × 100, where %T and %C are the percentages of aphids on treatment and control surface respectively. %FI, Percent feeding inhibition ± standard error.

When tested against the tick *Hyalomma lusitanicum*, Black pine EO showed a strong dose-dependant 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 the Mediterranean theileriosis in cattle [10] and their bites may cause bacterial infections [11]. To our knowledge this is the first report to that shows the acaricidal activity of black pine essential oil on *H. lusitanicum*. The monoterpene verbenol has been reported to have anti-ischemic and anti-inflammatory activity [12]. This compound has been also reported to as a repellant on *Ixodes ricinus* (L.) tick [13].

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

μg/mg Cellulose	Mortality (%)[a]					
	Douglas Fir	Silver Fir	Juniperus 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 Scheider-Orelli's formula.Values in the table represent the mean value of three replicates (±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		*M. javanica* J2 Mortality (%)[a] 1 μg/ml	0.5 μg/ml
Douglas fir		4.13±0.45	
Silver fir		1.71±0.46	
Juniperus 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 Scheider-Orelli's formula. Values are means (± standard error) of four replicates.

4. Conclusions

The major constituent of Black pine and Juniperus 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, while the nematicidial activity seems to be due to synergistic interaction of minor compounds rather than from the activity of the main compounds.

Author Contributions: A.G.-C. conceived the study; A.G.-C., M.F.A. and S.O. designed the methods; E.M. collected samples and performed the experiments; A.G.-C., M.F.A., S.O. and E.M. analyzed the data; A.G.-C. and S.O. contributed substantial resources and funding. E.M. wrote the manuscript's first draft, and all authors contributed to subsequent editions. All authors discussed the experiments and results and their interpretation. All authors have read and agreed to the published version of the manuscript.

Funding: This work has been partially supported by grant PID2019-106222RB-C31, MCI, Spain.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Garay, J.; Brennan, T.; Bon, D. Review: Essential oils a viable pest control alternative. *Int. J. Ecotoxicol. Ecobiol*. **2020**, *5*, 13–22. https://doi.org/10.11648/j.ijee.20200502.11.
2. Wajs-Bonikowska, A.; Sienkiewicz, M.; Stobiecka, A.; Maciąg, A.; Szoka, Ł.; Karna, E. Chemical composition and biological activity of *Abies alba* and *A. koreana* seed and cone essential oils and characterization of their seed hydrolates. *Chem. Biodivers.* **2015**, *12*, 407–418.
3. Tumen, I.; Hafizoglu, H.; Kilic, A.; Dönmez, I.E.; Sivrikaya, H.; Reunanen, M. Yields and constituents of essential oil from cones of *Pinaceae* spp. natively grown in Turkey. *Molecules* **2010**, *15*, 5797–5806.
4. Mamoci, E.; Mara, K.; Lloha, I.; Andrés, M.F.; Olmeda, S.; Gonzalez-Coloma, A. Chemical composition and bioactivity of essential oils from Albanian conifer plants. In Proceedings of the55thCroatian & 15thInternational Symposium on Agriculture. Vodice, Croatia, 16–21 January 2020; Mioč, B.,Širić, I., Eds.; Faculty of Agriculture, University of Zagreb: Zagreb, Croatia, 2020; pp. 213–217.
5. Burgueño-Tapia, E.; Castillo, L.; González-Coloma, A.; Joseph-Nathan, P. Antifeedant and phytotoxic activity of the sesquiterpene p-benzoquinone perezone and some of its derivatives. *J. Chem. Ecol.* **2008**, *34*, 766–771.
6. González-Coloma, A.; Sainz, P.; Olmeda, S.; Burillo, J.; Sanz, J.; Umpierrez, M.L.; Rossini, C. Desarrollo de Métodos de Bioensayos con Garrapatas Aplicados a la Detección de Potenciales Bioplaguicidas Botánicos; Echeverri, F., Rossini, C., Eds.; Universidad de Magallanes: Punta Arenas, Chile, 2013.

7. Andrés, M.F.; González-Coloma, A.; Sanz, J.; Burillo, J.; Sainz, P. Nematicidal activity of essential oils: A review. *Phytochem. Rev.* **2012**, *11*, 371–390.
8. Górski, R.; Tomczak, M. Usefulness of natural essential oils in the control of foxglove aphid (*Aulacorthum solani* Kalt.) occurring on eggplant (*Solanum melongena* L.). *Ecol. Chem. Eng. S.* **2010**, *17*, 345–349.
9. Yazdgerdian, A.R.; Akhtar, Y.; Isman, M.B. Insecticidal effects of essential oils against woolly beech aphid, *Phyllaphisfagi* (Hemiptera: Aphididae) and rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). *J. Entomol. Zool. Stud.* **2015**, *3*, 265–271.
10. Habela, M.; Rol, J.A.; Antón, J.M.; Peña, J.; Corchero, E.; Van Ham, I.; Jogejan, E. Epidemiology of Mediterranean theileriosis in Extremadura region, Spain. *Parasitologia* **1999**, *41*, 47–51.
11. Toledo, A.; Olmeda, A.S.; Escudero, R.; Jado, I.; Valcárcel, F.; Casado-Nistal, M.A.; Rodríguez-Vargas, M.; Gil, H.; Anda, P. Tick-borne zoonotic bacteria in ticks collected from central Spain. *Am.J. Trop. Med. Hyg.* **2009**, *1*, 67–74.
12. Choi, Y.; Lim, J.H.; Hwang, S.; Lee, J.C.; Cho, G.S.; Kim, W.K. Anti-ischemic and anti-inflammatory activity of (S)-cis-verbenol. *Free Radic. Res.* **2010**, *44*, 541–551.
13. Pålsson, K.; Jaenson, T.G.; Baeckström, P.; Borg-Karlson, A.K. Tick repellent substances in the essential oil of *Tanacetum vulgare*. *J. Med. Entomol*. **2008**, *45*, 88–93.
14. Ibrahim, S.K.; Traboulsi, A.F.; El-Haj, S. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita. Phytopathol. Mediterr*. **2006**, *45*, 238–246.