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Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers: Fr.

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Abstract

Essential oils of seven Moroccan Labiatae were chemically analysed by GC-MS and evaluated for their in vitro antifungal activity against *Botrytis cinerea*. Among them, *Origanum compactum* and *Thymus glandulosus* greatly inhibited the growth of the mycelium. The inhibition of *Botrytis cinerea* was 100% for both oils at 100 ppm, while the IC_{50}s were 35.1 and 79.2 ppm, respectively. *Mentha pulegium* exhibited moderate activity at 250 ppm since the inhibition of the mycelial growth was 58.5% and the IC_{50} was 233.5 ppm. The main constituents of the studied oils were also examined. Thymol and carvacrol that are the two main constituents of *Thymus glandulosus* and *Origanum compactum* exhibited the strongest antifungal activity with 100% of inhibition at 100 ppm, respectively.

Keywords: Moroccan Labiatae; GC-MS; antifungal activity; *Botrytis cinerea*

1. Introduction

Botrytis cinerea Pers: Fr. (grey mold rot) is a ubiquitous pathogen, which causes severe damage in many fruits, vegetables and ornamental crops in pre- and post-harvest. The pathogen infects leaves, stems, flowers and fruits. The grey mold disease is very destructive on crops under greenhouse conditions (Elad, 1997). The frequent applications of the most effective fungicides resulted in the selection and predominance of the pathogen resistant strains. Elad (1991) showed that *Botrytis cinerea* develops resistance against specific fungicides (benzimidazoles, dicarboximides, diethofuncarband and the sterol biosynthesis inhibitors) within a relatively short time. Additionally, many investigations have recently focused on alternatives to synthetic pesticides in order to comply with food safety standards. It is evident that most markets in industrialised countries are looking for chemical-free fresh or processed fruits and vegetables. Thus, several assays on the activity of essential oils against *Botrytis cinerea* have been published (Wilson et al., 1987; Shimoni, 1993; Arras et al., 1995; Carta et al., 1996; Cutler et al., 1996; Anthonov et al., 1997; Bhaskara Reddy et al., 1998).

In the prosecution of the valorisation of Moroccan aromatic plants used locally as remedies in folk medicine, we started a programme aimed at the evaluation of the antifungal role and other pharmacological properties of the volatile fractions from seven Moroccan plants in the hope of finding new natural products to be used in the biocontrol of plant diseases (El Mahi et al., 1998; Khalouki et al., 2000; Hmamouchi et al., 2001a,b; Lahlou et al., 2001a,b).

We report here the antifungal properties and the chemical composition of the essential oils of seven plants belonging to the Labiatae family. The antifungal activity of the main compounds of the studied essential oils was also investigated.

2. Materials and methods

2.1. Plant collection and essential oil isolation

Plants showing an aromatic character and belonging to Labiatae were collected in different regions of Morocco. They were taxonomically identified at the National Scientific Institute of Rabat (Department of Vegetable Biology, Laboratory of Botany). A voucher specimen of each sample

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Table 1
Chemical composition (%) of the oils of seven Moroccan Labiatae

Compounds	1	2	3	4	5	6	7
α-Thujene	0.5	0.4	0.3	0.8			
α-Pinene	2.2		0.5	0.4	15.3	5.1	0.9
Octen-3-ol			2.4	1.1			
Camphene		0.9	0.2		3.4	0.5	0.4
Actenol							
Nonane							
Sabinene	4.1	0.4				0.2	
β-Pinene	5.5	0.4	0.4	0.2	0.9	0.8	0.3
Myrcene	3.2		0.7	1.2	1.3	0.4	1.5
α-Phellandrene				0.1	2.1		
δ-3-Carene				0.1	0.3		
α-Terpinene				1.1	0.3		
p-Cymene		9.6		11.4	2.0	0.3	35.7
1,8-Cineole	36.6	2.0	0.3		11.6	1.2	0.5
Limonene	9.2		0.6	0.3	3.8		0.3
γ-Terpinene	0.3			7.1	0.3	0.4	2.0
Sabinene trans hydrate	0.4			0.6			
Terpinolene					0.7		
Mentthadiene-3,8-α-thujone			5.3				
Linalol oxyde		0.3		0.1			
Fenchone							
Linalol	0.2	28.9		2.0	5.4	23.0	1.4
β-Thujone							
Menthone	6.5						
Isomenthone	0.5						
p-Cymen-8-ol				0.1			
Myrtenal				1.9			
Chrysanthenone					0.9		
Camphre		2.3			11.6		0.1
Borneol					15.6		1.5
δ-Terpineol	3.5	1.4				0.3	0.2
Menthol	3.6						
Terpinene-4-ol	0.6	1.6		0.9			
Cetone							
Terpin-4-en-1-ol					0.9		
α-Terpineol	1.4			1.0	2.0		
Myrtenol				+			
Verbenone					11.2		
Isopulegol 1							
Isopulegol 2							
Pulegone	17.9		85.4				
Piperitenone			1.0				
Terpenyl acetate		0.9					
Bornyl acetate					1.4		1.3
Linalyl acetate		43.5				51.4	
Carvone							
Thymol	0.4	0.1		9.0		0.2	43.2
Carvacrol	0.4			58.1			1.7
Piperitone oxyde	0.6						
β-Caryophellene	0.7			1.6	0.9	0.9	0.4
Germacrene-D							
Bicyclogermacrene	0.4	2.0					
γ-Cadinene		2.4				0.3	
δ-Cadinene				0.1			
β-Bisabolene				0.1			
Spathulenol				0.6			
Caryophyllene oxyde		1.2			0.2	0.6	
Viridiflorol							
Total	98.7	98.3	97.1	99.9	92.6	85.6	91.4
Yield (%)	1.50	0.70	2.52	5.40	0.79	1.40	0.58
Native names	Mantha	Lkhzama	Fliyou	Zaater	Azir	Salmia	Zaater

+: presence of compound. 1: *Calamintha officinalis*; 2: *Lavendula dentata*; 3: *Mentha pulegium*; 4: *Origanum compactum*; 5: *Rosmarinus officinalis*; 6: *Salvia aegyptica*; 7: *Thymus glandulosus*.

was deposited in the herbarium of the Laboratory of Natural Products (Faculty of Medicine and Pharmacy of Rabat). All plants tested are presented in Table 1.

Air-dried, ground samples (200 g) were subjected to steam distillation for 2 h using a Clevenger-type apparatus recommended by the French Pharmacopoeia (1983). The oils were dried over anhydrous sodium sulfate and stored under refrigeration. The yields (w/w) were reported in Table 1. The oil was analysed in a Hewlett-Packard 5972 MS, fitted with a HP 5890 Series II GC and controlled by a G1034C Chemstation. A sample of 1 μl was injected under the following conditions: column—DB1 fused silica capillary column (20 m × 0.20 mm, film thickness 0.2 μm); carrier gas—helium (0.6 ml/min); injector temperature—250 °C; column temperature—50–250 °C at 3 °C/min; MS—electronic impact 70 eV. The identification of the compounds was achieved by comparing the retention times and the mass spectra with those of the standards included in the library (Stenhagen et al., 1974; Adams, 1989).

All the pure compounds of essential oils tested for their activity against *Botrytis cinerea* were purchased from Fluka.

2.2. Antifungal assay

Potato Dextrose Agar (PDA) (Merck) was autoclaved and cooled in a water bath to 40 °C, each oil or constituent was added to sterilised water at the concentration 1000 ppm and dissolved using ultrasounds homogeniser in ice back. The oil prepared as above was mixed with sterile molten PDA to obtain final concentrations 0, 10, 50, 100, 150, 200 and 250 ppm. The PDA was poured into petri dishes (≈20 ml/plate), which were then seeded with 5 mm diameter mycelial plug from edge of 7-day-old *Botrytis cinerea*. Plates in three replicates were used for each treatment. All plates were incubated in the dark at 24 °C for 7 days, time by which the growth of the control would have reached the edge of the plate. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control. The major essential oil components were screened in a similar manner. Their effects were compared to Procymidone, a fungicide belonging to the dicarboximide chemical group. Procymidone was used in a series of concentrations ranging from 0.001 to 10 ppm. The IC_{50} value was determined by the linear regression of the probit of the test fungus percentage inhibition and the log of the studied essential oil concentrations. The IC_{50} was the average of three replications. All the tests were repeated three times.

2.3. Statistical analysis

Newman–Keuls' test was undertaken using the procedure within the STATITCF statistical program.

3. Results and discussion

The results of GC-MS analyses are listed in Table 1. It can be seen that the monoterpens constitute the predominant class of compounds in *Calamintha officinalis* oil (94%), with 1,8-cineole (36.6%) being the main components, followed by pulegone (17.9%). The sample of *Lavendula dentata* oil contains linalyl acetate as a major component (43.5%) followed by linalol (28.9%), *p*-cymene (9.6%) and camphor (2.3%). The oil of *Mentha pulegium* was found to contain mainly pulegone (85.4%). For *Origanum compactum* oil, its major constituent is carvacrol (58.1%). While *Rosmarinus officinalis* essential oil was found to be rich in borneol (15.6%), α-pinene (15.3%), 1,8-cineole (11.6%), camphor (11.6%), verbenone (11.2%), *Salvia aegyptica* essential oil contains mainly linalyl acetate (51.4%), linalol (23.0%) and α-pinene (5.1%). The sample of *Thymus glandulosus* oil was found to be rich in thymol (43.2%) and *p*-cymene (35.7%).

To sum up, the present data reveals qualitative and quantitative variation in composition of the seven species of Labiatea. However, the GC-MS analyses show the predominance of the monoterpene hydrocarbons and the oxygenated monoterpene in all the oils under investigation.

In our study, we have found that among all the plant species tested, *Origanum compactum* and *Thymus glandulosus* have essential oils that show the greatest inhibition of mycelium growth of *Botrytis cinerea* at a very low concentration. The growth of the mycelium was completely

Table 2
Percentage of inhibition of *Botrytis cinerea* mycelium radial growth on PDA mixed with the essential oils

	Inhibition (%)								
	Concentration (ppm)								IC_{50} (ppm)
	0	0.1	10	50	100	150	200	250	
Calamintha officinalis	0.0	0.0	0.0	0.0 d	0.0 c	0.7 c	3.7 c	16.3 c	–
Lavendula dentata	0.0	0.0	0.0	0.0 d	0.0 c	0.0 c	0.0 d	1.9 e	–
Mentha pulegium	0.0	0.0	0.0	4.1 c	25.9 b	30.0 b	44.4 b	58.5 b	233.5 a
Origanum compactum	0.0	0.0	0.0	74.8 a	100 a	100 a	100 a	100 a	35.1 c
Rosmarinus officinalis	0.0	0.0	0.0	0.0 d	0.0 c	0.0 c	2.8 c	10.7 d	–
Salvia aegyptica	0.0	0.0	0.0	0.0 d	0.0 c	0.0 c	0.0 d	3.7 e	–
Thymus glandulosus	0.0	0.0	0.0	31.1 b	100 a	100 a	100 a	100 a	79.1 b

Means in the same column followed by the same letter are not significantly different according to the test of Newman–Keuls ($\alpha = 0.05$).

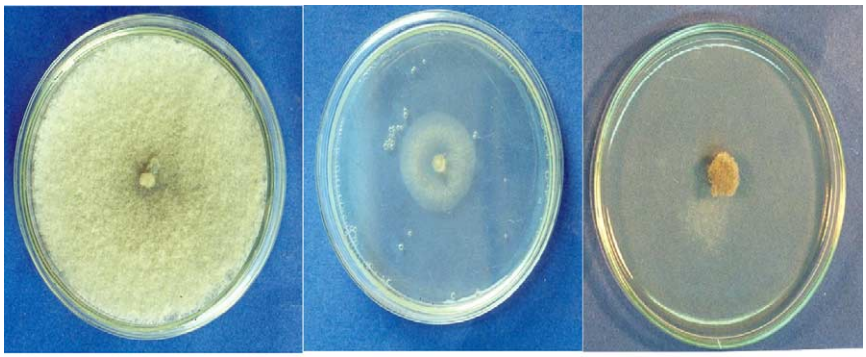

Fig. 1. Radial growth of *Botrytis cinerea* mycelium treated with *Origanum compactum* essential oil at 50 ppm (b) and 100 ppm (c) compared with the control (a) after 7 days at 24 °C.

inhibited at 100 ppm (Table 2; Fig. 1). The IC_{50}s for *Origanum compactum* and *Thymus glandulosus* were 35.1 and 79.2 ppm, respectively. *Mentha pulegium* showed a moderate activity at 250 ppm. Results in Tables 2 and 3 show that the inhibitory effect of the essential oils was mainly due to the two most abundant components, namely thymol and carvacrol. Furthermore, results indicate that the essential oils of *Origanum compactum* and *Thymus glandulosus* containing considerable amounts of thymol and/or carvacrol were strong inhibitors of *Botrytis cinerea*. This is in accordance with the finding of Bhaskara Reddy et al. (1998) who tested in vitro the essential oil of *Thymus vulgaris* (Laval-1 and Laval-2) for antifungal activity against *Botrytis cinerea* and *Rhizopus stolonifer*. Arras and Usai (2001) demonstrated that the antifungal activity of *Thymus capitatus* essential oil was due to carvacrol. Compared to Procymidone, the standard fungicide which is known to be very effective against *Botrytis cinerea* with IC_{50} is 0.2 ppm. It is interesting to note that the fungus was sensitive to carvacrol and thymol and the IC_{50}s were 18.6 and 18.9, respectively. Several authors have focused on the antimicrobial activity of thymol and carvacrol (Kim et al., 1995; Curtis et al., 1996). As to the mechanism of action of carvacrol, it has been suggested that it interacts with the cell membrane of the pathogen (Thompson, 1996). It has also been demonstrated that other species of *Thymus* and *Origanum* like *Thymus capitatus*, *Thymus zygis* and *Origanum syriacum* displayed excellent antifungal activities against *Botrytis cinerea* (Cutler et al., 1996; Arras and Usai, 2001). As shown in Tables 2 and 3, the percentage of inhibition due to the essential oils against the grey mold was dependent on the oil concentration: the more significant the decrease in the mycelial growth is, the higher the increase in the oils concentration. However, essential oils of *Calamintha officinalis*, *Rosmarinus officinalis*, *Mentha pulegium*, *Lavendula dentata* and *Salvia aegyptica*, the constituents of which are mainly borneol, *p*-cymene, linalyl acetate and pulegone, show slight or no inhibitory effect on the tested fungal organism (Table 2).

In conclusion, this study demonstrates the in vitro activity of essential oils of some Moroccan Labiatae and their constituents against the grey mold disease. However, further studies are required to determine the effect of these oils on spore germination and in vivo testing, in order to evaluate their potential as preventive treatments. Likewise, it would be recommended to check the effectiveness of thymol and carvacrol on resistant *Botrytis cinerea* strains and

Table 3
Percentage of inhibition of *Botrytis cinerea* mycelium radial growth on PDA mixed with the main constituents of the essential oils

	Inhibition (%)								
	Concentration (ppm)								IC_{50} (ppm)
	0	0.1	10	50	100	150	200	250	
α-Pinene	0.0	0.0 b	0.0 c	0.0 d	0.0 e	0.0 d	0.0 e	3.0 de	–
Borneol	0.0	0.0 b	0.0 c	0.0 d	6.7 d	7.4 c	7.4 d	9.3 cd	–
Carvacrol	0.0	0.0 b	30.0 a	79.6 b	100 a	100 a	100 a	100 a	18.6 a
Cineole	0.0	0.0 b	0.0 c	0.0 d	0.0 e	0.0 d	0.0 e	3.7 de	–
Linalool	0.0	0.0 b	0.0 c	0.0 d	0.0 e	0.0 d	7.4 d	12.6 c	–
Menthone	0.0	0.0 b	0.0 c	4.1 c	13.3 c	11.9 b	17.4 b	27.8 b	–
p-Cymene	0.0	0.0 b	0.0 c	0.0 d	0.0 e	0.0 d	0.0 e	3.7 de	–
R-(+)-Pulegone	0.0	0.0 b	0.0	0.0 e	0.0 d	0.0 d	12.6 c	30.4 b	–
Thymol	0.0	0.0 b	26.3 b	81.9 a	100 a	100 a	100 a	100 a	18.9 a
Procymidone (standard fungicide)	0.0	43.3 a	100 a	–	–	–	–	–	0.2 b

Means in the same column followed by the same letter are not significantly different according to the test of Newman–Keuls ($\alpha = 0.05$).

to determine the best time for harvesting the plants in order to obtain the highest level of activity.

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