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COMPOSITION AND *IN VITRO* ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS OF *Erigeron canadensis* AND *Myrtus communis* FROM FRANCE

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Essential oils of Erigeron canadensis *L. and* Myrtus communis *L. were tested in vitro as growth inhibitors against phytopathogenic fungi* Rhizoctonia solani *Kuhn,* Fusarium solani *(Mart.) Sacc. and* Colletotrichum lindemuthianum *(Sacc. & Magn.) Briosi & Cav. Both showed weak fungicidal acitivity, except the essential oil of M. communis that exerted a 60% growth inhibition against* R. solani *at a dose of 1600 ppm. Microscopic observation revealed that the essential oil of* M. communis *caused morphological alterations of hyphae of all fungi at 1600 ppm, while, at the same dose, only the hyphal morphology of* C. lindemuthianum *was affected by the essential oil of* Er. canadensis.

Key words: *Erigeron canadensis, Myrtus communis,* mycelial inhibition, phytopathogenic fungi.

During the last years there has been growing interest in testing natural compounds of different origins as defense for cultivated plants against phytopathogenic fungi [1]. In particular, essential oils were seen to exert good antifungal activities both *in vitro* and *in vivo* [2–9]. Although pharmacological activities of many essential oils have been described in the literature [10], their activity against phytopathogenic fungi has been less investigated. In the present work we wish to report the chemical composition and *in vitro* growth inhibition activity against three phytopathogenic fungi *Rhizoctonia solani* Kuhn, *Fusarium solani* (Mart.) Sacc. and *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav. of essential oils of *Erigeron canadensis* L. and *Myrtus communis* L. collected in France.

The chemical composition of essential oils of *Er. canadensis* and *M. communis* are reported in Tables 1 and 2 respectively. The essential oil of *Er. canadensis* contained 18 compounds, limonene being the main one (76.03%). The essential oil of *M. communis* contained 14 compounds, α-pinene and 1,8-cineol (eucalyptol) together representing around 86%.

Data reported in Tables 3 and 4 revealed that *R. solani* growth is slightly inhibited only at the highest dose (1600 ppm) using the essential oil of *Er. canadensis*, while growth inhibition of this fungus reached 28% at a dose of 800 ppm using the essential oil of *M. communis* and became doubled (60%) by doubling the dose of the oil. *F. solani* growth was very little inhibited (4.50%) using the essential oil of *Er. canadensis* at a dose of 400 ppm, but increasing the dose up to 1600 ppm increased the percentage of inhibition slightly (12.71%). The same pattern was recorded for *F. solani* using the essential oil of *M. communis*, the highest percentage of growth inhibition being 15.59% at 1600 ppm. *C. lindemuthianum* growth inhibition was 18.75% at a 400 ppm dose of the essential oil of *Er. canadensis* and increased up to 29.27% at 1600 ppm. Treatment with the essential oil of *M. communis* resulted in little growth inhibition (21.41%) at 1600 ppm, while lower doses favored the growth of *C. lindemuthianum* cultures, although in a very low percentage (2.56%); a similar effect was already observed using other essential oils [4]. At lower doses, both essential oils did not modify the morphological features of fungi cultures, as seen by microscopic observations. At higher doses (1600 ppm) the morphology of hyphae changed: in particular, using the essential oil

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TABLE 1. Retention Indices (R_i) and Percentage Chemical Composition of Essential Oil of *E. canadensis*

Compounds	R_i	%*
α-Pinene	942	Tr.
β-Pinene	978	1.57±0.06
β-Myrcene	988	3.62±0.04
Cosmene	1001	0.32±0.04
Limonene	1025	76.03±0.07
Δ^3-Carene	1027	3.87±0.03
Thujone	1100	1.70±0.04
Camphor	1126	0.39±0.06
Isoborneol	1149	Tr.
Menthol	1168	0.23±0.05
Isobornyl acetate	1279	0.17±0.05
β-Caryophyllene	1428	2.13±0.05
Epi-bicyclosesquiphellandrene	1431	0.34±0.06
α-Santalene	1435	5.84±0.04
Germacrene D	1468	0.16±0.04
α-Cariophyllene	1469	1.50±0.05
β-Sesquiphellandrene	1472	0.35±0.02
Germacrene B	1477	1.78±0.07

*Values expressed as mean of 3 measurements ± S.D.

TABLE 2. Retention Indices (R_i) and Percentage Chemical Composition of Essential Oil of *M. communis*

Compounds	R_i	%*
α-Pinene	942	52.90±0.07
β-Pinene	978	0.66±0.05
Iso-butyl *iso*-butyrate	989	0.64±0.07
1,8-Cineol	1017	32.92±0.04
4-Carene	1043	0.79±0.02
Linalool	1097	4.21±0.03
Terpinen-4-ol	1129	0.42±0.03
α-Terpineol	1178	2.46±0.06
Linalyl butyrate	1325	0.52±0.06
α-Terpineol acetate	1333	0.64±0.05
Geranyl acetate	1363	1.64±0.04
β-Cariophyllene	1428	1.33±0.04
Germacrene D	1468	0.33±0.05
α-Cariophyllene	1469	0.54±0.02

*Values expressed as mean of 3 measurements ± S.D.

of *Er. canadensis* resulted in alterations of hyphae of *C. lindemuthianum*, which appeared dichotomously branched in the apical portion. Treatment with the essential oil of *M. communis* resulted in structural damages of hyphae of *R. solani*, although the diameter of treated hyphae did not change when compared to that of control ones (7.16 mm and 7.15 mm respectively); hyphae of *F. solani* appeared more thick and corrugated than untreated ones, which appeared to grow linearly; hyphae of *F. solani* and of *C. lindemuthianum* appeared more branched and irregularly shaped than untreated ones.

TABLE 3. Percentage of Growth Inhibition of the Tested Phytopathogenic Fungi Treated with Different Concentrations of Essential Oil of *Er. canadensis*

Dose (ppm)	% growth inhibition*		
	R. solani	*F. solani*	*C. lindemuthianum*
100	n.d.	n.d.	n.d.
400	n.d.	4.50±2.03	18.75±7.37
800	n.d.	8.53±1.79	22.10±3.60
1600	22.35±3.63	12.71±1.28	29.27±1.22

*Values expressed as mean of 8 measurements ± S.D.
n.d. = Not detected.

TABLE 4. Percentage of Growth Inhibition of Phytopathogenic Fungi Treated with Different Concentrations of Essential Oil of *M. communis*

Dose (ppm)	% growth inhibition*		
	R. solani	*F. solani*	*C. lindemuthianum*
100	n.d.	1.76±0.59	-2.56±3.45
400	n.d.	1.18±1.57	4.43±1.16
800	28.09±2.47	2.55±0.90	13.12±3.17
1600	60.00±2.84	15.59±0.42	21.41±1.04

*Values expressed as mean of 8 measurements ± S.D.
n.d. = Not detected.

The essential oils of *Er. canadensis* and *M. communis* were shown to exert growth inhibition activity against three phytopathogenic fungi, *R. solani* Kuhn, *F. solani* (Mart.) Sacc. and *C. lindemuthianum* (Sacc. & Magn.) Briosi & Cav., although the observed activities were less than those reported for the essential oils of thyme and mint against the same fungi [8]. *C. lindemuthianum* was shown to be more susceptible towards the essential oil of *Er. canadensis*: in this case growth inhibition was accompanied by deep modification of hyphae morphology. *R. solani* was the more susceptible strain towards the essential oil of *M. communis*, revealing a good percentage (60%) of growth inhibition at higher doses. Moreover, also in this case, marked modifications in hyphae morphology were detected: they appeared, collapsed probably due to dehydration induced by the oil components. Similar morphological modifications were reported for the same fungus using the essential oil of thyme. The fungicidal activity of essential oils of *Er. canadensis* and *M. communis* may be due to their main components, limonene, α-pinene, and 1,8-cineol, respectively. In fact, the antifungal properties of these monoterpenes have been well documented in the literature [3, 7]. Studies of the *in vivo* properties of the essential oils from *Er. canadensis* and *M. communis* against phytopathogenic fungi are now in progress in our laboratories.

EXPERIMENTAL

The chemical composition of each oil was determined by GC-MS analysis using a Hewlett Packard 6890 gas chromatography equipped with a 12.5 m × 0.25 mm MetSil column coupled to HP ChemStation Software. The carrier gas was helium at a pressure of 3.5 kg/cm^2 and the column temperature was programmed from 50°C to 270°C at 4°C/min. The chromatogram was obtained using a reporting integrator and the composition recorded as percent area. Mass spectra were obtained from a GC-MS system, operating in the EI mode at 70 eV, equipped with a 12.5 m × 0.25 mm MetSil column and an

HP 5973 Mass Selective Detector, using the same chromatographic conditions reported above. The column was connected to the mass spectrometer ion source *via* an open split interface heated at 250°C. Identification of chemical constituents was based on a comparison of their retention indices (R_i) [11] and mass spectra obtained from commercially available samples and from the Nist98 Mass Spectral Database. *Rhizoctonia solani* Kuhn strains were obtained from thyme seedlings, *Fusarium solani* (Mart.) Sacc. strains were obtained from the CBS culture collection (Utrecht, The Netherlands) no. 231.31, and *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav. strains were isolated from bean plants. All fungi were cultivated in agar-potato culture medium (PDA, Difco). Antifungal activity was assayed in 8.5 cm diameter Petri dishes containing a PDA culture medium. The essential oil of *Er. canadensis* was used as 50% v/v solution in absolute ethanol; the essential oil of *M. communis* was used as 12.5% v/v solution in 80% ethanol [12]. Each solution was added to the PDA culture medium at 40–45°C then put in Petri dishes. Essential oils were assayed at doses of 100, 400, 800, and 1600 ppm and 5 Petri dishes were treated twice with each dose level. PDA culture mediums containing only ethanol were used as controls. Fungi were collected from the external border of active growing cultures and inoculated in the form of a 5 mm diameter disc in each dish. Petri dishes were incubated at 22 ± 1°C. Fungal growth was detected 2, 5, 7, and 10 days after inoculation by measuring the diameters of colonies. The antifungal activity of essential oils was measured at the highest mycelar growth stage of cultures in control dishes and calculated as percentage of mycelar growth inhibition as reported by Pandey and coworkers [5]. Morphological variations of cultures were detected 7 days after inoculation by microscopic observation (Leitz Laborlux 12 optical microscope), and images were taken by a JVC camera connected to a personal computer. The diameters of the hyphae were calculated by an Axio.Vision 2.05 (Zeiss) computer program.

Antifungal activity of each oil is reported in Tables 3 and 4.

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