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Antifungal activity of essential oils of three aromatic plants from western Algeria against five fungal pathogens of tomato (*Lycopersicon esculentum* Mill)

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Summary

The antifungal effect of the essential oils from *Thymus capitatus* L., *Daucus crinitus* Desf. and *Tetraclinis articulate* Vahl., aerial parts was evaluated in vitro against five phytopathogenic fungi of tomato (*Fusarium oxysporum*, *Alternaria solani*, *Aspergillus niger*, *Penicillium sp1* and *Penicillium sp2*). Our results showed that among the three plant species tested, *T. capitatus* oil was the most potent antifungal against the fungi (inhibition of mycelial growth of 100 % at a concentration of 2 µg mL⁻¹). Furthermore, the essential oil of *T. articulata* was also effective against *F. oxysporum*, *A. solani*, *A. niger*, *Penicillium sp1* and *Penicillium sp2* with an inhibition of mycelial growth greater than 57 % at a concentration of 5 µg mL⁻¹. *D. crinitus* essential oil was less effective. *T. capitatus* essential oil was dominated by carvacrol (69.6 %) and p-cymene (12.4 %). The isochavicol isobutyrate (44.9 %) and isochavicol 2-methylbutyrate (9.7 %) were the major compounds in *D. crinitus* essential oil, while the most abundant compounds in *T. articulata* were α-pinene (32.0 %), cedrol (11.0 %) and 3-carene (9.6 %). The plant essential oils were found to be an effective antifungal against mycelial growth and, therefore, can be exploited as an ideal treatment against disease rot of tomato or as a new potential source of natural additives for the food and/or pharmaceutical industries.

Introduction

Tomato (*Lycopersicon esculentum*) is an important commercial crop in the world. Nutritional values of tomato make it a widely accepted vegetable by consumers. Nevertheless, tomato is a very perishable vegetable with a short shelf-life and high susceptibility to fungal disease. Tomatoes are among the most popular fruits grown in Algeria. They are of an excellent quality and are greatly appreciated for their nutritional value. Furthermore, tomato production represents an important agricultural and economic activity in the country. The growing awareness of consumers concerning the relation between food and health is revolutionizing the food industry. Fungal pathogens are mainly responsible for the decay of fruits and vegetables during the postharvest period (PATHAK, 1997). *Aspergillus*, *Fusarium* and *Penicillium* are responsible for spoilage of many foods and causes decay on stored fruits damaged by insects, animals, early splits, and mechanical harvesting. Apart from causing diseases in plants, many species of *Aspergillus*, *Penicillium* and *Alternaria* can also synthesize mycotoxins (AGRIOS, 1997; ROJAS et al., 2005). Considerable interest has developed on the preservation of foods by the use of essential oils to effectively retard growth and mycotoxin production. Essential oils and their main components possess a wide spectrum of biological activity, which may be of great importance in several fields, from food chemistry to pharmacology and pharmaceuticals (CRISTANI, 2007). The main aim of this work was to evaluate the antifungal properties of the essential oils of *T. capitatus*,

D. crinitus and *T. articulata* against phytopathogens that cause severe diseases in tomato, such as *F. oxysporum*, *A. solani*, *A. niger*, *Penicillium sp1* and *Penicillium sp2*.

Materials and methods

Plant materials and essential oils extraction

Aerial parts of *D. crinitus* were collected in Bensekrane forest area (Tlemcen Province) at the flowering stage, in June 2011. The oil yield was 0.37 % (w/w). *T. capitatus* aerial parts were collected from Beni snous in Tlemcen city at the flowering stage, during June 2011 and yielded 0.52 % (w/w). *T. articulata* aerial parts were collected from Oujlida region, Tlemcen Province during June 2011 and yielded 0.31 % (w/w). The plant species were stored at -18 °C after harvest. A portion (550-600 g) of material from each plant species was subjected to a Clevenger-type apparatus according to the European Pharmacopoeia (EUROPEAN PHARMACOPOEIA, 2004). The essential oils were dried over anhydrous sodium sulfate and, after filtration, stored in sterilized amber vials at 4 °C until it was used.

Gas chromatography

Analyses were carried out using a Perkin Elmer Clarus 600 GC apparatus equipped with a dual flame ionization detection system and 2 fused-silica capillary columns (60 m x 0.22 mm I.D., film thickness 0.25 µm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). The oven temperature was programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. Injector and detector temperatures were maintained at 280 °C. Essential oils were injected in the split mode (1/50), using helium as the carrier gas (1 mL/min); the injection volume was 0.2 µL. Retention indices (RI) of the compounds were determined from Perkin-Elmer software.

Gas chromatography-mass spectrometry

Essential oils were analyzed with a Perkin-Elmer TurboMass quadrupole analyzer, coupled to a Perkin-Elmer Autosystem XL, equipped with 2 fused-silica capillary columns and operated with the same GC conditions described above, except for a split of 1/80. Electronic Impact (EI) mass spectra were acquired under the following conditions: Ion source temperature 150 °C, energy ionization 70 eV, mass range 35-350 Da (scan time: 1 s).

Component identification

The identification of the components was based on a comparison: (i) between the calculated retention indices on the polar (RI p) and apolar (RI a) columns with those of pure standard authentic compounds and literature data (JENNINGS and SHIBAMOTO, 1980; KÖNIG et al., 2001; NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY,

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2008); and (ii) of the mass spectra with those of our own library of authentic compounds and with those of a commercial library (MC LAFFERTY and STAUFFER, 1994; MC LAFFERTY and STAUFFER, 1988; NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY, 1999).

Component quantification

Quantification of the essential oil components was carried out using the methodology reported by COSTA et al. (2008), and modified as follows. The response factor (RF) of 29 standard compounds grouped into 7 chemical groups (monoterpene hydrocarbons, sesquiterpene hydrocarbons, alcohols, ketones, aldehydes, esters, and others) was measured using GC (ZNINI et al., 2011). RFs and calibration curves were determined by diluting each standard in hexane at 5 concentrations, containing tridecane (final concentration = 0.7 g/100 g) as an internal standard. Analysis of each standard was performed in triplicate. For the quantification of the essential oil components, tridecane (0.2 g/100 g) was added as internal standard to the essential oil. The correction factor (average of the response factors from standards) of each chemical group was calculated and used to determine the essential oil component concentration (g/100 g) according to the chemical group.

Pathogenic fungi

Fusarium oxysporum, *Alternaria solani*, *Aspergillus niger*, *Penicillium sp1* and *Penicillium sp2* were isolated from naturally decayed tomato after storage of several weeks. These isolates were the most aggressive one in our collection and produced the largest lesions on inoculated fruit. A pure culture of these fungus were maintained on potato dextrose agar medium (PDA: potato 200, dextrose 20 g and agar 15 gL⁻¹ in distilled water at 25 °C) in the presence of a quantity of lactic acid (25 %) for stop the growth of bacteria. The plates were incubated at 25 ± 2 °C for 8 days and darkness. The developing fungal colonies were purified and identified up to the species level by microscopic examination through the help of the following references (BARNETT and HUNTER, 2006).

In vitro antifungal assay

The antifungal activity of the three essential oils was tested using radial growth technique (BAJPAI et al., 2007). Appropriate volumes of the stock solutions of the oils in dimethyl sulfoxide (DMSO) were added to PDA medium immediately before it was poured into the Petri dishes (9.0 cm diameter) at 40-45 °C to obtain two concentrations (2.0 and 5.0 µg mL⁻¹). Each concentration was tested in triplicate. Parallel controls were maintained with DMSO mixed with PDA. The discs of mycelial felt (0.5 cm diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the centre of Petri dishes. Carbendazim was used as reference fungicide. The treatments were incubated at 27 °C in the dark. Colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. Percentage of mycelial growth inhibition was calculated from the

formula: $(I\%) = [(DC-DT)/DC] \times 100$ (PANDEY et al., 1982);

where DC and DT are average diameters of fungal colony of control and treatment, respectively.

Statistical Analysis

The inhibitory effect of essential oils on mycelial growth was expressed as mean ± standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's t-test. The separation of means

was done by using the least significant difference test at $p < 0.05$. Analysis of each test was performed in triplicate.

Results

Essential oils composition

A total of 26 components accounting to 99.5 % of the essential oil composition of *T. capitatus* were identified by comparison of their EI-mass spectra and their retention indices (RI) with those of our own authentic compound library (Tab. 1). The essential oil was highly dominated by oxygenated compounds (87.1%) with high amount of aromatic terpenic components (82.6 %). However, hydrocarbons appeared also in appreciable proportion (12.4%) which monoterpene hydrocarbons are well represented (10.7 %). Indeed, the main constituents of essential oil were carvacrol (69.6 %), p-cymene (12.4 %) followed by γ-terpinene (4.3 %), myrcene (2.1 %), α-terpinene (1.7 %), linalool (1.7 %) and terpinen-4-ol (1.1 %). These results were in accordance with those previously reported in literature (AMARTI et al., 2008; BOUNATIROU et al., 2007; RUBERTO et al., 2000; TAWAHA and HUDAIB, 2012). Other hand, various chemical profiles of essential oils (thymol, cavacrol or thymol/cavacrol as main components) have been reported according to geographical origins of *T. capitatus* (KAROUSO et al., 2005; MICELI et al., 2006). The analysis of the essential oil from the aerial parts of *D. crinitus* harvested in the forest of Bensekrane (Tlemcen) identified 30 components, which accounted for 91.3 % of the total composition. Their retention indices and relative percentages are shown in Tab. 1. The main components of the aerial parts oil were phenylpropanoids isochavicol esters, principally the isochavicol isobutyrate (44.9 %). The other major components identified were: isochavicol 2-methylbutyrate (9.7 %), pentadecane (5.1 %) and undecane (4.1 %) (Tab. 1). This result is in according with literature data (LANFRANCHI et al., 2010).

A total of 54 components accounting for 95.9 % of the total oil of *T. articulata* were identified (Tab. 1). The essential oils was highly dominated by the monoterpene hydrocarbons (63.8 %) followed by oxygenated sesquiterpenes (14.7 %) and sesquiterpene hydrocarbons (10.5 %). However, the oxygenated monoterpenes appeared in small proportion (6.4 %). The most abundant compounds were α-pinene (32.0 %), cedrol (11.0 %), 3-carene (9.6 %), limonene (4.3 %), sabinene (4.3 %) and (E)-β-caryophyllene (4.0 %). BEN JEMIA et al. (2013) have isolated and identified, by GC-MS, 66 constituents, the major constituents of the oil are: bornyl acetate (31.4 %), α-pinene (24.5 %) and camphor (20.3 %). while TOUMI et al. (2011) have identified, by GC/MS, more 45 compounds, with camphor(23.4-31.6 %), bornyl acetate (17.1-25.8 %), borneol (6.6-14.3 %), limonene (3.70-10.1 %) and α-pinene (6.5-11.3 %) were the major components of *T. articulata* essential oil. It was observed that the percentage of α-pinene (19.8-24.9 %) and bornyl acetate (40.2-59.2 %) for the leaves oils from two different sites in Algeria were the major constituents (CHIKHOUNE et al., 2013). In addition, the percentage of cedrol and 3-carene found in our essential oil was higher than cedrol and 3-carene in previous studies. Generally, the quality and quantity of components available in essential oils may be affected by several factors, such as plant genotype, geographical condition, season, and agronomic condition (GUMUS et al., 2010).

Antifungal activity of three essential oils against the development of fungi of tomato

The data presented in Tab. 2 show the antifungal activity of 3 plant species, belonging to 3 botanical families (Lamiaceae, Apiaceae and Cupressaceae), against *F. oxysporum*, *A. solani*, *A. niger*, *Penicillium sp1* and *Penicillium sp2*. The effect of plant essential oils varied according to plant species. Indeed, 2 plant species out of 3 reduced

Tab. 1: Chemical compositions of aerial parts essential oils of *T. capitatus*, *D. crinitus* and *T. articulate*.

No. ^a	Components	RI _a ^b	RI _a ^c	RI _p ^d	<i>T. capitatus</i>	<i>D. crinitus</i>	<i>T. articulate</i>	Identification ^e
	Nonane	906	902	907	-	0.6	-	RI, MS
	α -Thujene	932	924	1028	0.2	-	tr	RI, MS
	α-Pinene	936	931	1028	0.9	0.5	32.0	RI, MS
	α -Fenchene	941	943	1039	-	-	0.6	RI, MS
	Camphene	950	945	1071	0.2	-	0.3	RI, MS
	Oct-1-en-3-ol	962	962	1441	0.5	-	-	RI, MS
	Sabinene	973	967	1122	-	0.6	4.3	RI, MS
	β -Pinene	978	972	1113	0.1	0.1	1.4	RI, MS
	Myrcene	987	982	1160	2.1	0.6	3.3	RI, MS
	α -Phellandrene	1002	999	1161	0.2	-	1.5	RI, MS
	3-Carene	1005	1006	1149	0.1	-	9.6	RI, MS
	α -Terpinene	1008	1011	1270	1.7	-	-	RI, MS
	p-Cymene	1015	1015	1270	12.4	0.2	0.5	RI, MS
	Limonene	1025	1023	1201	-	0.9	4.3	RI, MS
	β -Phellandrene	1023	1023	1209	-	-	1.4	RI, MS
	(E)- β -Ocimene	1041	1037	1247	-	0.6	0.7	RI, MS
	(Z)- β -Ocimene	1029	1022	1234	0.6	-	-	RI, MS
	γ -Terpinene	1051	1050	1245	4.3	1.6	0.7	RI, MS
	(E)-Sabinene hydrate	1051	1054	1445	0.1	-	0.2	RI, MS
	Nonanal	1076	1074	1403	-	0.1	-	RI, MS
	Terpinolene	1082	1079	1281	0.2	0.4	3.2	RI, MS
	(Z)-Sabinene hydrate	1087	1084	1537	-	-	0.8	RI, MS
	Linalool	1083	1085	1538	1.7	0.2	0.2	RI, MS
	Undecane	1100	1098	1101	-	4.1	-	RI, MS
	3-Octyl acetate	1113	1107	1330	-	-	0.2	RI, MS
	Veratol	1112	1113	1713	-	-	0.1	RI, MS
	Camphor	1123	1124	1506	0.1	-	-	RI, MS
	(Z)-Verbenol	1027	1128	1642	-	-	0.3	RI, MS
	Isoborneol	1143	1144	1670	0.5	-	-	RI, MS
	Borneol	1148	1150	1688	0.3	-	-	RI, MS
	Terpinen-4-ol	1164	1162	1591	1.1	0.1	2.0	RI, MS
	α -Terpineol	1176	1176	1690	0.1	-	0.1	RI, MS
	Octyl acetate	1188	1187	1460	-	2.3	-	RI, MS
	Decanal	1188	1187	1483	-	1.4	-	RI, MS
	Linalyl acetate	1239	1239	1552	-	-	0.2	RI, MS
	Decanol	1263	1259	1729	-	0.1	-	RI, MS
	Nonanoic acid	1263	1263	2119	-	0.1	-	RI, MS
	(E)-Anethole	1264	1261	1815	-	-	0.1	RI, MS
	Thymol	1266	1263	2181	0.6	-	-	RI, MS
	Bornyl acetate	1269	1269	1562	-	-	0.7	RI, MS
	Carvacrol	1278	1286	2193	69.6	-	-	RI, MS
	Eugenol	1330	1329	2164	0.1	-	-	RI, MS
	α -Terpinyl acetate	1335	1333	1686	-	-	1.8	RI, MS
	(E)-Myrtanyl acetate	1366	1370	1479	-	-	0.1	RI, MS
	β -Bourbonene	1386	1384	1518	-	-	0.1	RI, MS
	β -Elemene	1389	1386	1584	-	-	0.2	RI, MS
	Dodecanal	1389	1389	1695	-	3.1	-	RI, MS

No. ^a	Components	RI _a ^b	RI _a ^c	RI _p ^d	<i>T. capitatus</i>	<i>D. crinitus</i>	<i>T. articulata</i>	Identification ^e
	β-Funebrene	1419	1411	1591	-	-	1.6	RI, MS
	(E)-β-Caryophyllene	1421	1416	1591	1.6	0.6	4.0	RI, MS
	Thujopsene	1435	1427	1614	-	-	0.2	RI, MS
	α-Humulene	1455	1448	1668	0.1	-	2.5	RI, MS
	α-Acoradiene	1444	1455	1616	-	-	0.1	RI, MS
	β-Acoradiene	1458	1459	1642	-	-	0.1	RI, MS
	Alloaromadendrene	1462	1461	1630	-	-	0.1	RI, MS
	γ-Curcumene	1475	1471	1680	-	-	0.3	RI, MS
	Germacrene D	1479	1474	1700	-	-	1.3	RI, MS
	β-Selinene	1482	1480	1703	-	-	0.1	RI, MS
	γ-Humulene	1483	1480	1702	-	0.7	-	RI, MS
	Pentadecane	1500	1497	1502	-	5.1	-	RI, MS
	δ-Cadinene	1520	1511	1760	-	0.1	0.3	RI, MS
	β-Elemol	1535	1533	2063	-	-	0.4	RI, MS
	Isochavicol isobutyrate	1546	1541	2134	-	44.9	-	RI, MS
	Dodecanoic acid	1554	1560	2474	-	1.1	-	RI, MS, ref
	Caryophyllene oxide	1578	1567	1969	0.1	-	0.4	RI, MS
	Dodecyl acetate	1585	1580	1882	-	2.5	-	RI, MS, ref
	Globulol	1589	1577	2085	-	-	0.9	RI, MS
	Cedrol	1595	1591	2101	-	-	11.0	RI, MS
	Humulene epoxide II	1602	1599	2044	-	-	0.1	RI, MS
	epi-Cedrol	1613	1614	2141	-	-	0.2	RI, MS
	α-Acorenol	1623	1617	2106	-	-	0.3	RI, MS
	γ-Eudesmol	1619	1624	2198	-	-	0.1	RI, MS
	τ-Cadinol	1633	1632	2146	-	-	0.2	RI, MS
	α-Eudesmol	1632	1636	2211	-	-	0.3	RI, MS
	Isochavicol 2-methyl butyrate	1651	1648	2255	-	9.7	-	RI, MS
	Bulnesol	1665	1664	2198	-	-	0.2	RI, MS
	Heptadecane	1700	1703	1699	-	3.4	-	RI, MS
	Tetradecanoic acid	1761	1756	2649	-	3.1	-	RI, MS, ref
	Cedryl acetate	1764	1750	2160	-	-	0.6	RI, MS
	Neophytadiene	1807	1807	1918	-	0.4	-	RI, MS, ref
	Hexadecanoic acid	1951	1949	2916	-	1.1	-	RI, MS
	Manool	2070	2109	2684	-	-	0.3	RI, MS
	(E)-Phytol	2114	2102	2620	-	1.7	-	RI, MS
	Total identification %				99.5	92.0	96.5	
	% Hydrocarbon compounds				12.4	20.5	74.5	
	% Monoterpene hydrocarbons				10.7	5.5	63.8	
	% Sesquiterpene hydrocarbons				1.7	1.4	10.7	
	% Non terpenic hydrocarbon compounds				-	13.2	-	
	% Diterpenes hydrocarbons				-	0.4	-	
	% Oxygenated compounds				87.1	71.5	22.0	
	% Oxygenated monoterpenes				3.8	0.3	6.5	
	% Oxygenated sesquiterpenes				0.1	-	14.7	
	% Non terpenic oxygenated compounds				0.5	14.9	0.2	
	% Aromatic compounds				82.6	-	0.1	
	% Phenylpropanoids				0.1	54.6	-	
	% Oxygenated diterpenes				-	1.7	0.5	

^a Order of elution is given on apolar column (Rtx-1), ^b Retention indices on the apolar Rtx-1 column (RIa), ^c Retention indices on the polar Rtx-Wax column (RIp), ^d Retention indices on the polar Rtx-Wax column (RIp), ^e RI: Retention Indices; MS: Mass Spectrometry in EI mod.

Tab. 2: Percentage of inhibition of mycelial growth at various volumes of essential oils.

Incubation	<i>F. oxysporum</i> 25°C ± 2	<i>A. solani</i> 25°C ± 2	<i>A. niger</i> 25°C ± 2	<i>Penicillium</i> sp1 25°C ± 2	<i>Penicillium</i> sp2 25°C ± 2
Essential oil (2 µg mL ⁻¹)					
<i>T. capitatus</i>	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
<i>T. articulata</i>	36.11 ± 0.08	35.12 ± 0.01	11.11 ± 0.11	34.56 ± 0.02	45.12 ± 0.06
<i>D. crinitus</i>	-	-	-	-	54.32 ± 0.21
Essential oil (5 µg mL ⁻¹)					
<i>T. articulata</i>	72.22 ± 0.06	70.12 ± 0.20	57.77 ± 0.11	64.44 ± 0.12	84.44 ± 0.08
<i>D. crinitus</i>	-	-	-	5.55 ± 0.21	77.77 ± 0.06

mycelial growth of *F. oxysporum*, *A. solani*, *A. niger*, *Penicillium* sp1 and *Penicillium* sp2 by more than 50 %. Among these plants *T. capitatus*, belonging to the families of Lamiaceae, completely inhibited mycelial growth of tested fungus. *T. capitatus* essential oil produced the greatest reduction in mycelium growth with these fungi at 2 µg mL⁻¹, with percentage reductions of 100 % (Tab. 2). The second most effective essential oil with this five fungi was *T. articulata* essential oil, with percentage of mycelial reduction in *F. oxysporum*, *A. solani*, *A. niger*, *penicillium* sp1 and *penicillium* sp2 of 36.11, 35.12, 11.11, 34.56 and 45.12 %, respectively, at the same concentration (Tab. 2). However, the data indicate that the percentage inhibition of mycelial growth increased with increasing concentration of essential oils for all strains tested, suggesting that the essential oil of *T. articulata* inhibited the growth of all strains in a dose-dependent manner. Essential oil *D. crinitus* cause no percentage of mycelial reduction, except against *penicillium* sp2. This activity was more pronounced, where the percentage of inhibition increased to 54.32 % at 2 µg mL⁻¹, reaching a maximum of 77.77 % at 5 µg mL⁻¹, suggesting that this strain was the most sensitive to the essential oil (Tab. 2).

Discussion

In this study, the antifungal activity of essential oils of three plant species was evaluated against *F. oxysporum*, *A. solani*, *A. niger*, *Penicillium* sp1 and *Penicillium* sp2. The mycelial growth of colonies in the presence of the essential oil of *T. capitatus* and *T. articulata* showed that it effectively controlled all the fungi tested. The mycelial growth of colonies in the presence of the essential oil of *T. capitatus* and *T. articulata* showed that it effectively controlled all the fungi tested. This efficiency can be explained by the presence of active molecules that inhibited the growth of the five phytopathogenic fungi. This activity may be produced by a single major compound or by the synergistic or antagonistic effect of various compounds (DEBA et al., 2008). Several authors have attributed the antifungal capacity of plant essential oils to the presence of components such as phenolic and terpene compounds (BEUCHAT, 1994; DAVIDSON, 1997; NYCHAS, 1995) indicated that mycelial growth inhibition is caused by the monoterpenes present in essential oils. These components would increase the concentration of lipidic peroxides such as hydroxyl, alkoxy and alko peroxyl radicals and so bring about cell death. However, the influence of essential oil or bioactive compounds on flavor and aroma of tomato was not investigated and further work should be conducted to purpose the use efficiency of oil components in real applications such as fumigant. In conclusion, this paper is a part of an overall study that aims to determine the antifungal activities of natural floral resources of Algeria, in order to find new bioactive natural products. The essential oils of these plants

studied, exhibited an interesting antifungal activity against mycelial growth. Further work is necessary to explore the efficacy of these essential oils against disease rot of tomato and to exploit these oils as a new potential source of natural additives for the food and/or pharmaceutical industries.

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