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Antibacterial Activity of *Cuminum cyminum* L. and *Carum carvi* L. Essential Oils

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Essential oils extracted by hydrodistillation from fruits of *Cuminum cyminum* L. and *Carum carvi* L. were analyzed by gas chromatography (GC) and GC-mass spectrometry (MS). The main components of *C. cyminum* oil were *p*-mentha-1,4-dien-7-al, cumin aldehyde, γ-terpinene, and β-pinene, while those of the *C. carvi* oil were carvone, limonene, germacrene D, and *trans*-dihydrocarvone. Antibacterial activity, determined with the agar diffusion method, was observed against Gram-positive and Gram-negative bacterial species in this study. The activity was particularly high against the genera *Clavibacter*, *Curtobacterium*, *Rhodococcus*, *Erwinia*, *Xanthomonas*, *Ralstonia*, and *Agrobacterium*, which are responsible for plant or cultivated mushroom diseases worldwide. In general, a lower activity was observed against bacteria belonging to the genus *Pseudomonas.* These results suggest the potential use of the above essential oils for the control of bacterial diseases.

KEYWORDS: ***Cuminum cyminum* L.; *Carum carvi* L.; Apiaceae; plant extracts; essential oils; natural bactericides; plant bacterial disease control; mycopathogens**

INTRODUCTION

Control of plant bacterial diseases remains difficult due to the limited availability of bactericides. Only a few chemical products are available, and their use is hampered by limited efficacy in the field but mainly for their potential negative effects either in the environment or with human and animal health. The use of antibiotics in plant protection is limited because of the possibility to select pathogen populations resistant to bactericides and the potential transfer of resistant genes to animal and human pathogenic bacteria (*1*). This matter is still an object of debate although the use of antibiotics is forbidden in many European Union countries. Only the United States and a few other countries allow the use of oxytetracycline and streptomycin for the control of bacterial diseases on important crops (*1*). The use of copper compounds, which are widely used for the control of plant bacterial diseases, will be limited in the European Union countries by rule 473/2002 due to their impact on the environment. As a consequence, measures to control plant bacterial diseases are mostly limited to prevention. Agronomic practices that minimize initial infection and dissemination of bacterial pathogens between plants and fields are very useful although poorly effective under high disease pressure. Healthy propagation materials (i.e., seeds, etc.) are an effective measure to limit disseminating casual agents over limited or long distances (*2*). Unfortunately, the availability of healthy seed is often unreliable due to the ineffectiveness of seed certification agencies to eradicate plant pathogens prior to infection or in contaminated seed lots. This is particularly true for plant pathogenic bacteria. Sanitation methods used to eradicate bacteria from seed include physical procedures (i.e., hot water, dry heat, etc.); chemical treatments with $Ca(OCl)_2$, NaOCl, or HCl and organic acids (i.e., acetic acid). The efficacy of these treatments may be poor or have adverse effects on seed germinability (*2*). The availability of new and ecocompatible bactericides may be very useful for bacterial control of diseases in the field and for seed treatments. Essential oils are known for their antimicrobial capability (*3*) and have the potential to control plant diseases caused by bacteria and, in particular, eradicate bacteria from seeds. Research on the use of essential oils to inhibit bacterial growth is very limited. The first report of Maruzzella et al. (*4*) is followed by limited reports (*5*−*11*) on the action of essential oils for the control of plant pathogenic bacteria.

Cumin (*Cuminum cyminum* L.) and caraway (*Carum carvi* L.) are aromatic plants included in the Apiaceae family and are used to flavor foods, added to fragrances, and for medical preparations. In particular, *C. carvi* essential oil is used in liqueurs, mouthwashes, toothpastes, soaps, and perfumes. In addition, *C. cyminum* and *C. carvi* are used as antispasmodic, carminative, and appetite stimulant agents (*12*).

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Table 1. Bacterial Strains Used in This Study[a]

bacteria	strains
Gram-Negative	
Escherichia coli	ITM103
Pseudomonas syringae pv. *phaseolicola*	NCPPB2571, IPV-BO1917, USB316, USB320
P. syringae pv. *pisi*	NCPPB3496, 895-A
P. syringae pv. *syringae*	Y37, NCPPB1910, B366
P. syringae pv. *aptata*	NCPPB2664, NCPPB872
P. syringae pv. *apii*	NCPPB1626
P. syringae pv. *atrofaciens*	NCPPB2612, GSPB1742
P. syringae pv. *lachrymans*	USB326, USB327
P. syringae pv. *maculicola*	NCPPB2038, NCPPB2704
P. syringae pv. *tomato*	USB328, USB329
P. syringae pv. *glycinea*	NCPPB2752, NCPPB2753
P. cichorii	ICMP5707
P. viridiflava	DPP5, DPP18
P. corrugata	NCPPB2445
P. tolaasii	NCPPB2192
P. reactans	NCPPB1311
P. agarici	NCPPB2289
Erwinia carotovora subsp. *carotovora*	ICMP5702
E. carotovora subsp. *atroseptica*	ICMP1526
E. herbicola	ICMP9900
Agrobacterium tumefaciens	USB1001, USB1005
Burkholderia gladioli pv. *agaricicola*	ICMP11096
Ralstonia solanacearum[b]	FC486
Xanthomonas campestris pv. *phaseoli*	NCPPB3035, GSPB1217, ICMP238
X. campestris pv. *phaseoli* var. *fuscans*[b]	ICMP239, ICMP3403, GSPB275, XCPFu4487
X. campestris pv. *vesicatoria*	NCPPB422, DAPP-PG95, DAPP-PG32, DAPP-PG35
X. campestris pv. *campestris*	NCPPB528
Gram-Positive	
Bacillus megaterium	ITM100
Clavibacter michiganensis subsp. *michiganensis*	DPP2, DPP3
C. michiganensis subsp. *sepedonicus*	NCPPB2137
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	ICMP2584, ICMP5370
C. flaccumfaciens pv. *betae*	NCPPB372, NCPPB374
Rhodococcus fascians	NCPPB2551, NCPPB3067

[a] ITM, Istituto Tossine e Micotossine (Bari, Italy); NCPPB, National Collection Plant Pathogenic Bacteria (United Kingdom); IPV-BO, Istituto di Patologia Vegetale (Università di Bologna, Italy); USB, Università degli Studi della Basilicata (Potenza, Italy); GSPB, Gottinger Sammlung Phitopathogener Bakterien (Gottingen, Germany); ICMP, International Collection of Microrganism from Plants (Auckland, New Zealand); DPP, Dipartimento di Protezione delle Piante (Università della Tuscia, Viterbo, Italy); DAPP-PG, Dipartimento di Arboricoltura e Protezione delle Piante (Università degli Studi di Perugia, Italy). [b] Bacterial strains FC486 and XCPFu4487 were supplied by Dr. N. Schaad (USDA-ARS-FDWSRU-Bacteriology, Fort Detrick, MD) and Dr. L. E. Claflin (Department of Plant Pathology, Kansas State University, Kansas), respectively.

In our research, essential oils were extracted from the fruits of *C. cyminum* and *C. carvi* and the resulting extracts were evaluated by gas chromatography (GC) and GC-mass spectrometry (MS) analysis and then assayed in vitro for their capability to inhibit the growth of plant pathogenic bacteria (*13*) and bacteria responsible for diseases on cultivated mushrooms (*14*). Preliminary results of this study were previously reported (*15*).

MATERIALS AND METHODS

Bacterial Cultures. Bacterial strains used in this study, maintained under lyophilized conditions at 4 °C, are listed in **Table 1**. Subcultures were obtained by growing bacteria for 48−72 h on King's medium B (KB) (*16*) for pseudomonads and on WA (sucrose, 10 g/L; bactopeptone, 5 g/L; K_2HPO_4, 0.5 g/L; $MgSO_4 \times 7H_2O$, 0.25 g/L; and agar, 18 g/L) (*17*) for other bacterial genera.

Isolation and Analysis of Essential Oils. Aliquots (25 g) of *C. cyminum* and *C. carvi* dry fruits were ground, and the resulting powder was hydrodistillated for 3 h following a previous procedure (*18*). Prior to use, the essential oils were stored in sealed vials under N_2 at 4 °C and were analyzed by GC and GC-MS as described by Senatore and Rigano (*19*).

The oil components were identified by calculating their Kovats indices in relation to a homologous series of *n*-alkanes (C_8-C_{22}) under the same conditions (*20*), comparing mass spectra with those reported in the literature (*21*, *22*) and in the GC-MS computer database (NIST 98 and Wiley-5). Furthermore, the identity of some of the oil components was confirmed by GC analysis by coinjection with authentic substances. The compounds were as follows: (*E*)-anethole (Fluka, 10370); caryophyllene (Sigma-Aldrich, C9653); carvacrol (Sigma, 28,219-7); carveol (Sigma, 19,238-4); carvone (Sigma, 12,493-1); *p*-cymene (Sigma, C12,145-2); eugenol (Fluka, 46100); geranyl acetate (Fluka, 45897); linalool (Fluka, 62140); myrcene (Aldrich, 10,0005); nonanal (Sigma, N3,080-3); octanal (Sigma, O-560-8); α-phellandrene (Fluka, 77429); α-pinene (Aldrich, 14,752-4); *β*-pinene (Aldrich, 42,016-6); *γ*-terpinene (Aldrich, 22,318-2); and tricyclene (Fluka, 91485). The component relative concentrations in each essential oil were calculated based on GC peak areas without using correction factors.

Disk Diffusion Assay. Ten microliters of a 1:1 serial dilution of each essential oil in 80% (v/v) methanol and 1.6 mg/mL of rifampicin were added to 6 mm diameter sterile blank disks (Oxoid S.p.A., Milan, Italy). These were placed on the surface of Petri plates containing either 10 mL of KB or WA (0.7% agar) depending on the bacterial species. Aliquots of the target bacterial suspensions were added to the media, maintained at 45 °C to obtain a final population of about 10^7 cfu/mL. After 48 h of incubation at 25 °C, the minimal inhibitory quantity (MIQ), which causes an apparent inhibition zone around the 6 mm diameter disks, was recorded. The assays were performed twice with three replicates.

RESULTS AND DISCUSSION

Results of GC-MS analyses of *C. cyminum* and *C. carvi* essential oils (**Table 2**) showed that the chemical compositions

Table 2. Chemical Composition of *Cuminum cyminum* (A) and *Carum carvi* (B) Essential Oils

component	KI[a]	composition (%) A	B
tricyclene[b]	925	0.1	
α-pinene[b]	936	0.6	
sabinene	974	0.5	
β-pinene	977	11.4	
myrcene[b]	991	0.9	
octanal[b]	1002		1.2
α-phellandrene[b]	1011	1.3	
o-cymene	1026	3.1	
p-cymene[b]	1036	5.7	
limonene	1039	3.1	18.2
β-phellandrene	1039	2.2	
γ-terpinene[b]	1068	12.8	
nonanal[b]	1103		0.3
linalool[b]	1108		0.3
cis-limonene oxide	1135		tr
trans-limonene oxide	1140		0.1
(*Z*)-tagetone	1154		0.2
dihydrocarveol	1190		4.5
cis-dihydrocarvone	1192		0.4
trans-dihydrocarvone	1199		14.0
trans-carveol[b]	1218		0.1
cis-carveol[b]	1229		0.1
cumin aldehyde	1239	16.1	
carvone[b]	1245		23.3
cumin alcohol	1251	0.4	
(*Z*)-2-decenal	1251		0.4
p-mentha-1,3-dien-7-al	1256	8.7	
(*E*)-2-decenal	1260		0.2
cis-carvone oxide	1268		0.3
p-mentha-1,4-dien-7-al	1280	27.4	
(*E*)-anethole[b]	1282		3.3
perillaldehyde	1291	0.6	
perilla alcohol	1299	0.3	
carvacrol[b]	1302		6.7
eugenol[b]	1355	0.7	
geranyl acetate[b]	1379	1.7	
caryophyllene[b]	1414	1.3	6.1
germacrene D	1472		16.2
δ-cadinene	1524		0.5
germacrene B	1556		3.8

[a] KI, Kovats index on DB-5 column; tr, trace (<0.5%). [b] Substance identification was confirmed by GC analysis by coinjection with authentic substances.

Table 3. MIQ (μg) of *C. cyminum* and *C. carvi* Essential Oils Against Various Gram-Negative and Gram-Positive Bacteria

bacteria	no. of strains	MIQ (μg)[a] *C. cyminum*	*C. carvi*
Gram-Negative			
E. coli	1	3680	910
P. syringae pv. *phaseolicola*	4	2760	1820
P. syringae pv. *pisi*	2	5520	5460
P. syringae pv. *syringae*	3	1840	1820
P. syringae pv. *aptata*	2	7360	2730
P. syringae pv. *apii*	1	1840	1820
P. syringae pv. *atrofaciens*	2	920	910
P. syringae pv. *lachrymans*	2	920	910
P. syringae pv. *maculicola*	2	2760	2730
P. syringae pv. *tomato*	2	7360	5460
P. syringae pv. *glycinea*	2	920	910
P. cichorii	1	7360	7280
P. viridiflava	2	Na	Na
P. corrugata	1	7360	910
P. tolaasii	1	920	910
P. reactans	1	3680	3640
P. agarici	1	3680	910
E. carotovora subsp. *carotovora*	1	1840	910
E. carotovora subsp. *atroseptica*	1	460	455
E. herbicola	1	3680	3640
A. tumefaciens	2	690	682.5
B. gladioli pv. *agaricicola*	1	7360	3640
R. solanacearum	1	230	227.5
X. campestris pv. *phaseoli*	3	575	170.2
X. campestris pv. *phaseoli* var. *fuscans*	4	460	455
X. campestris pv. *vesicatoria*	4	345	227.5
X. campestris pv. *campestris*	1	920	455
Gram-Positive			
B. megaterium	1	920	455
C. michiganensis subsp. *michiganensis*	2	133.4	255.7
C. michiganensis subsp. *sepedonicus*	1	460	455
C. flaccumfaciens pv. *flaccumfaciens*	2	460	455
C. flaccumfaciens pv. *betae*	2	460	682.5
R. fascians	2	460	910

[a] MIQ, average quantity needed for the bacterial growth inhibition. The MIQ was calculated by considering the average densities of 0.92 and 0.91 g/mL for *C. cyminum* and *C. carvi* essential oils, respectively. Na, the deposition of 8 μL of essential oils on sterile blank disks did not lead to an inhibition zone.

of the two oils were totally different. The main components of *C. cyminum* oil were *p*-mentha-1,4-dien-7-al (27.4%), cumin aldehyde (16.1%), γ-terpinene (12.8%), and β-pinene (11.4%), whereas those of the *C. carvi* oil were carvone (23.3%), limonene (18.2%), germacrene D (16.2%), and *trans*-dihydrocarvone (14.0%).

The assays for antibacterial activity against Gram-positive and Gram-negative bacteria of *C. cyminum* and *C. carvi* essential oils showed an antibacterial activity against all bacterial strains used in this study except strains of *Pseudomonas viridiflava*, which were resistant to the oils even to 8 μL, the highest quantity used in the assays (**Table 3**). Strains of the same bacterial species, with a few exceptions, showed a similar sensitivity to the oils. The antibacterial activity of the two essential oils was relatively high, and the most sensitive Gram-negative bacteria were those of the genera *Erwinia*, *Agrobacterium*, *Ralstonia*, and *Xanthomonas*, all important plant pathogens (**Table 3**). Quantities of oil less than 1 μL were capable of inhibiting growth. Sensitivity to both oils was shown by Gram-positive bacteria belonging to the genera *Clavibacter*, *Curtobacterium*, and *Rhodococcus*. Pseudomonads were generally more resistant. Nevertheless, 1 μL of both essential oils was sufficient to inhibit the growth of strains of *P. syringae* pv. *atrofaciens*, *P. syringae* pv. *lachrymans*, *P. syringae* pv. *glycinea*, and *P. tolaasii*. Our laboratory strains, *Escherichia coli* and *Bacillus megaterium*, were sensitive to both oils, and results were similar to the phytopathogenic bacteria.

In parallel antimicrobial assays, the MIQ of rifampicin on bacterial strains used in this study was between 1 and 4 μg for fluorescent pseudomonads and lower than 1 μg with *Xanthomonas campestris* pv. *phaseoli* and Gram-positive bacteria (**Table 4**). Comparison between activity of rifampicin and crude essential oils showed a similar behavior for either *C. cyminum* or *C. carvi* essential oils. In fact, the same effect was observed with 920−1840 and 455−920 μg of the *C. cyminum* and *C. carvi* essential oils when assayed against the above bacteria, respectively.

The MIQ, expressed in μg, was calculated by considering the average densities of 0.92 and 0.91 g/mL of *C. cyminum* and *C. carvi* oils, respectively. The above values were obtained by weighting 100 μL oil samples. At least three determinations were performed.

The antimicrobial activity of the essential oils is attributed to those known main components and the resulting synergistic or antagonistic action. However, minor components may also contribute to the biological activity. The antibacterial activity of *C. cyminum* essential oil is perhaps attributable to the high level of cumin aldehyde (16.1%), a compound with known

Table 4. MIQ (μg) of Rifampicin and *C. cyminum* and *C. carvi* Essential Oils on Selected Gram-Positive and Gram-Negative Phytopathogenic and Mycopathogenic Bacteria

strains	MIQ (μg)[a]		
	rifampicin	*C. cyminum*	*C. carvi*
P. syringae pv. *phaseolicola* NCPPB2571	1	1840	1820
P. syringae pv. *syringae* B366	2	1840	1820
P. syringae pv. *atrofaciens* NCPPB2612	2	920	910
P. syringae pv. *lachrymans* 442	1	920	910
P. syringae pv. *glycinea* NCPPB2752	4	920	910
P. tolaasii NCPPB 2192	2	920	910
X. campestris pv. *phaseoli* NCPPB3035	0.0156	920	455
X. campestris pv. *phaseoli* var. *fuscans* ICMP239	0.031	460	455
C. michiganensis subsp. *michiganensis* DPP2	0.031	460	455
C. michiganensis subsp. *sepedonicus* NCPPB2137	<0.0156	460	455
C. flaccumfaciens pv. *flaccumfaciens* ICMP5370	0.125	460	455
C. flaccumfaciens pv. *betae* NCPPB372	0.0625	460	455

[a] MIQ, average quantity needed for the bacterial growth inhibition. The MIQ was calculated by considering the average densities of 0.92 and 0.91 g/mL for *C. cyminum* and *C. carvi* essential oils, respectively.

antimicrobial properties (*23*, *24*), and to β-pinene, the other main component (11.4%) of *C. cyminum* essential oil, which inhibited the growth of bacteria (*7*, *25*). Limonene (3.1%), geranyl acetate (1.7%), eugenol (0.7%), α-pinene (0.6%), perillaldehyde (0.6%), and sabinene (0.5%), minor components of *C. cyminum* essential oil, are known bactericides (*7*, *25*, *26*) and may contribute to antimicrobial activity.

The antibacterial activity of *C. carvi* essential oil is apparently due to carvone (23.3%), limonene (18.2%), carvacrol (6.7%), and linalool (0.3%), which inhibit the growth of fungi and bacteria (*7*, *23*−*28*).

In general, results of this study confirmed the antimicrobial activity of essential oils on microorganisms responsible for human and animal disease (*29*, *30*), those responsible for food spoilage (*31*, *32*), and phytopathogenic bacteria and fungi (*5*, *9*−*11*, *33*). *C. cyminum* and *C. carvi* essential oils inhibited the growth of *Aspergillus parasiticus* (*23*) and yeasts and Gram-positive and Gram-negative bacteria (*34*). Our research reveals the bactericide activity of the above oils against plant pathogenic bacteria including those pathogenic on cultivated mushrooms.

Essential oils or their components appear promising for possible use as bactericides for the control of plant bacterial diseases. Furthermore, of particular interest is the possibility of these compounds for seed treatments against phytopathogenic bacteria to partially prevent long distance dissemination.

The significant antibacterial activity of essential oils against bacterial pathogens of mushrooms appears promising as a control protocol. Other studies are necessary to evaluate the possible toxicity of essential oils to seeds, plants, and mushrooms. Appropriate formulations will also be required. Inhibition of seed germination by several essential oil components was previously reported (*35*−*38*) and was attributed to the lipophilic nature of oils (*37*). This may not be the general mechanism since some highly lipophilic components, such as limonene and α-pinene, of essential oils were demonstrated to exhibit minimal activity on seed germination (*37*). Inhibition of seed germination by essential oils or their compounds may enhance other desirable features as in the case of carvone, which is used as a reversible suppressant of sprouting in stored potatoes (*27*).

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