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Antibacterial Activity of *Coriandrum sativum* L. and *Foeniculum vulgare* Miller Var. *vulgare* (Miller) Essential Oils

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Essential oils were extracted from the fruits of *Coriandrum sativum* L. and *Foeniculum vulgare* Miller var. *vulgare* (Miller) and assayed in vitro for antibacterial activity to *Escherichia coli* and *Bacillus megaterium*, bacteria routinely used for comparison in the antimicrobial assays, and 27 phytopathogenic bacterial species and two mycopathogenic ones responsible for cultivated mushroom diseases. A significant antibacterial activity, as determined with the agar diffusion method, was shown by *C. sativum* essential oil whereas a much reduced effect was observed for *F. vulgare* var. *vulgare* oil. *C. sativum* and *F. vulgare* var. *vulgare* essential oils may be useful natural bactericides for the control of bacterial diseases of plants and for seed treatment, in particular, in organic agriculture. The significant antibacterial activity of essential oils to the bacterial pathogens of mushrooms appears promising.

KEYWORDS: **Natural bactericides; bacterial disease control; plant pathogenic bacteria; mycopathogens; plant extracts; essential oils; *Coriandrum sativum*; *Foeniculum vulgare* var. *vulgare***

INTRODUCTION

Agriculture production incurs yearly losses because of plant diseases. Control is mainly achieved by using pesticides, which may be toxic to applicators and consumers and which may be harmful to the environment. Furthermore, pathogens often become resistant to pesticides (*1*).

The control of bacterial diseases of crops is a considerable problem due to the limited availability of bactericides and the ability of a large number of phytopathogenic bacteria to spread, even at long distances, by contaminated and/or infected seeds (*2*). Besides the antibiotic and copper compounds, which to some extent present undesirable attributes for either human and animal health or the environment, no other active principles are available for bacterial plant disease control. Furthermore, antibiotics oxytetracycline and streptomycin, used on plants in United States, are actually forbidden in the agricultural practices in many countries to avoid the possible selection of individuals resistant to the drugs and hence the possible horizontal genetic transfer to animal and human pathogens. Although the above matter is an object of debate, as a matter of a fact, the selection of strains resistant to oxytetracycline is apparently rare but the streptomycin resistant population of *Erwinia amylovora*, *Pseudomonas* spp., and pathovars of *Xanthomonas campestris*, causal agents of important plant diseases, has been ascertained (*1*). A consequence of the above effect was the failure of disease control. In addition, the use of copper compounds, because of their general toxicity and mainly because of the impact they have on the environment, is on its way to being restricted and controlled in the European Union through rule 473/2002.

In the last years, the biological proprieties of plant extracts have been reevaluated (*3*), and many studies have pointed out on the possibility to use the essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of microorganisms pathogenic to consumers and/or responsible for food spoilage.

The request of a reduction in the use of the pesticides in agriculture prompts the need for the development of alternative active compounds, possibly harmless to the consumers and to the environment and useful for the protection of crops from parasites and/or methods for the control of plant bacterial diseases to be used in integrated crop management (*4*, *5*) as well as in bioorganic agriculture.

In the last years, after the first studies of Maruzzella et al. (*6*), studies to ascertain the growth inhibition of plant pathogenic bacteria by plant extracts have been intensified (*7*−*12*), and the results strongly suggested the potential use of essential oils for the control of plant bacterial diseases.

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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
Agrobacterium tumefaciens	USB1001, USB1005
Burkholderia gladioli pv. *agaricicola*	ICMP 11096
Xanthomonas campestris pv. *phaseoli*	NCPPB3035, GSPB1217, ICMP238
X. campestris pv. *phaseoli* var. *fuscans*	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 flaccunfaciens pv. *flaccunfaciens*	ICMP2584, ICMP5370
C. flaccunfaciens pv. *betae*	NCPPB372, NCPPB374
Rhodococcus fascians	NCPPB2551, NCPPB3067

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

The aromatic proprieties of the fruits of coriander (*Coriandrum sativum* L.) and wild fennel [*Foeniculum vulgare* Miller var. *vulgare* (Miller)], plants belonging to the family *Apiaceae* typical of the Mediterranean region, are exploited by the pharmaceutical industry to correct the flavor of medicines and, above all, of some laxatives of plant origin. In the case of fennel oil, an effect on the digestive processes has been ascertained; hence, it may exert an additive effect (*13*).

The objective of this study was to evaluate the antibacterial activity in vitro of coriander and fennel essential oils toward bacteria responsible for diseases on plants and cultivated mushrooms (*14*). Preliminary results of this study have been previously reported (*15*).

MATERIALS AND METHODS

Bacterial Cultures. Bacterial strains used in this study are reported in **Table 1**. Strains were lyophilized and stored at 4 °C for long-term storage. The laboratory strains of *Escherichia coli* and *Bacillus megaterium* were used for comparison. Subcultures were obtained by growing bacteria for 48−72 h on King medium B (KB) (*16*) for pseudomonads and on WA (sucrose, 10 g/L; bacto-peptone, 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 the other bacterial species.

Extraction and Analysis of Essential Oils. Aliquots of 25 g of dried fruits of *C. sativum* and *F. vulgare* var. *vulgare* were ground, and the resulting powder was subjected to hydrodistillation for 3 h following the procedure previously reported (*18*). The essential oils were stored in sealed vials under N_2 at 4 °C and analyzed by gas chromatography (GC) and GC/mass spectrometry (MS) as previously described (*19*).

The oil components were identified by calculating their Kováts indices in relation to a homologous series of *n*-alkanes (C_8−C_{22}) under the same conditions (*20*) and by 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 used as follows: *trans*-anethole (Fluka, 10370); camphor (Fluka, 21310); caryophyllene (Sigma-Aldrich, C9653); caryophyllene oxide (Aldrich, 36199-2); estragole (Aldrich, A2920-8); eugenol (Fluka, 46100); fenchone (Serva, 21328 and 21329); geraniol (Serva, 2235); geranyl acetate (Fluka, 45897); linalool (Fluka, 62140); myrcene (Aldrich, 100005); nerol (Serva, 30280); α-phellandrene (Fluka, 77429); α-pinene (Aldrich, 14752-4); *β*-pinene (Aldrich, 42016-6); α-terpinene (Aldrich, 22318-2); *γ*-terpinene (Fluka, 86478); terpineol (Fluka, 86480); tricyclene (Fluka, 91485); and thymol (Sigma, T-0501). The component relative concentrations in each essential oil were calculated based on GC peak areas without using correction factors.

Disk Diffusion Assay. Ten microliter 1:1 serial dilutions in methanol of stock solutions of each essential oil, obtained in 80% (v/v) methanol, and of 1.6 mg/mL of rifampicin solution, a pure bactericide used for comparison, were added to 6 mm diameter sterile blank disks (Oxoid S.p.A., Milan, Italy) previously deposited onto the surface of Petri plates containing 10 mL of KB or WA (0.7% agar) depending on the bacterial species. Aliquots of target bacteria suspensions were added to the above 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

Table 2. Chemical Composition of *C. sativum* (A) and *F. vulgare* Var. *vulgare* (B) Essential Oils

component	KI[a]	composition (%) A	B
tricyclene[b]	925		tr
α-tujene	928	tr	
α-pinene[b]	936	5.1	2.7
camphene	953	0.8	0.2
sabinene	974	tr	0.1
β-pinene[b]	977	0.5	tr
myrcene[b]	991	0.8	tr
α-phellandrene[b]	1011		0.5
α-terpinene[b]	1017	tr	
p-cymene	1036	6.3	2.0
1,8-cineole	1036	0.1	
limonene	1039	3.6	15.0
β-phellandrene	1039		2.3
γ-terpinene[b]	1068	0.3	0.1
fenchone[b]	1090		12.6
terpinolene	1092		0.2
linalool[b]	1108	64.5	
camphor[b]	1156	6.4	
borneol	1168	tr	
α-terpineol[b]	1188	0.8	
estragole[b]	1196		0.9
fenchyl acetate	1221		0.2
nerol[b]	1226	4.6	
geraniol[b]	1251	0.4	
(*E*)-2-decenal	1261	0.1	
geranial	1266	tr	
(*Z*)-anethole	1269		0.5
(*E*)-anethole[b]	1282	0.3	59.2
thymol[b]	1298	0.1	
eugenol[b]	1355	0.2	0.3
geranyl acetate[b]	1379	0.4	
caryophyllene[b]	1414	tr	
caryophyllene oxide[b]	1569	tr	
apiole	1679		0.1

[a] KI, Kováts index in DB-5 column; tr, trace (<0.05%). [b] Substances identification was confirmed by GC analysis by coinjection with authentic substances.

6 mm diameter disks, was recorded. The assays were performed twice with three replicates.

RESULTS

Analysis of Essential Oils Composition. The relative amounts (%) of the components of *C. sativum* and *F. vulgare* var. *vulgare* essential oils, calculated based on GC peak areas, are reported in **Table 2** according to their retention indices on a DB-5 column. The main components of the two oils were different, but some of the minor components were present, although at different levels, in both preparations. In particular, the main components of *C. sativum* and *F. vulgare* var. *vulgare* essential oils were linalool (64.5%) and (*E*)-anethole (59.2%), respectively.

Antibacterial Activity of Test Oils. The oils showed an antibacterial activity toward the majority of the bacterial strains used in this study, but in general, the bactericidal activity of *C. sativum* oil was higher than that of *F. vulgare* var. *vulgare*. In particular, coriander oil inhibited the growth of *E. coli* and *B. megaterium*. Furthermore, the above oil inhibited the growth of strains of important plant pathogenic bacteria belonging to either Gram-negative genera such as *Pseudomonas*, *Erwinia*, *Xanthomonas*, and *Agrobacterium* or Gram-positive genera such as *Clavibacter*, *Curtobacterium*, and *Rhodococcus* (**Table 3**). The only exceptions were those strains tested of *Pseudomonas syringae* pv. *lachrymans*, *Pseudomonas viridiflava*, and *Pseudomonas reactans*, which were apparently resistant since at least

Table 3. MIQ (μg) of *C. sativum* (A) and *F. vulgare* Var. *vulgare* (B) Essential Oils against Bacteria

bacteria	no. of strains	MIQ (μg)[a] A	B
Gram-negative			
Escherichia coli	1	870	NA
Pseudomonas syringae pv. *phaseolicola*	4	2610	NA
P. syringae pv. *pisi*	2	2610	NA
P. syringae pv. *syringae*	3	3480	NA
P. syringae pv. *aptata*	2	3480	NA
P. syringae pv. *apii*	1	ND	NA
P. syringae pv. *atrofaciens*	2	6960	3840
P. syringae pv. *lachrymans*	2	NA	NA
P. syringae pv. *maculicola*	2	870	NA
P. syringae pv. *tomato*	2	3480	NA
P. syringae pv. *glycinea*	2	870	960
P. cichorii	1	6960	NA
P. viridiflava	2	NA	NA
P. corrugata	1	3480	NA
P. tolaasii	1	NA	7680
P. reactans	1	NA	NA
P. agarici	1	3480	NA
Erwinia carotovora subsp. *carotovora*	1	435	7680
E. carotovora subsp. *atroseptica*	1	435	7680
Agrobacterium tumefaciens	2	435	2880
Burkholderia gladioli pv. *agaricicola*	1	3480	7680
Xanthomonas campestris pv. *phaseoli*	3	217.5	480
X. campestris pv. *phaseoli* var. *fuscans*	4	217.5	720
X. campestris pv. *vesicatoria*	4	217.5	1440
X. campestris pv. *campestris*	1	217.5	5760
Gram-positive			
Bacillus megaterium	1	435	NA
Clavibacter michiganensis subsp. *michiganensis*	2	374.1	7680
C. michiganensis subsp. *sepedonicus*	1	435	960
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	2	435	NA
C. flaccumfaciens pv. *betae*	2	652.5	NA
Rhodococcus fascians	2	435	1920

[a] MIQ, average quantity needed for the bacterial growth inhibition. The MIQ was calculated by considering the average densities of 0.87 and 0.96 g/mL for *C. sativum* and *F. vulgare* var. *vulgare* essential oils, respectively. NA, the deposition of 8 μL of essential oils on sterile blank disks did not lead to an inhibition zone; ND, not determined.

the deposition of 8 μL of essential oils, the higher quantity used in the assays, on the disks did not lead to any inhibition zone.

The MIQ of rifampicin, as determined against representative bacterial strains, was between 1 and 4 μg for fluorescent pseudomonads and less than 1 μg for *X. campestris* pv. *phaseoli* and the Gram-positive bacteria belonging to *Clavibacter michiganensis* subspecies and *Curtobacterium flaccumfaciens* pathovars (**Table 4**). The same effect was observed with 870−6960 and 217.5−435 μg of the *C. sativum* essential oil when assayed against the above bacteria, respectively.

The MIQ, expressed in μg, was calculated by considering the average densities of 0.87 and 0.96 g/mL of *C. sativum* and *F. vulgare* var. *vulgare* oils, respectively. The above values were obtained by weighting 100 μL of oil samples. At least three determinations were performed.

DISCUSSION

Previous studies have already shown the growth inhibition activity on different microorganisms either by *C. sativum* (*23*) or *F. vulgare* essential oils (*24*). However, this is the first time that the bactericide activity of the above oils has been demonstrated toward plant pathogens as well as bacteria pathogens on cultivated mushrooms.

The results of this study confirm the antimicrobial activity of the plant extracts and/or essential oils as already reported

Table 4. MIQ (μg) of Rifampicin and *C. sativum* (A) and *F. vulgare* Var. *vulgare* (B) Essential Oils on Selected Gram-Positive and Gram-Negative Phytopathogenic Bacteria

bacteria	MIQ (μg)		
	rifampicin	A	B
Pseudomonas syringae pv. *phaseolicola* NCPPB2571	1	1740	NA
P. syringae pv. *syringae* B366	2	3480	NA
P. syringae pv. *atrofaciens* NCPPB2612	2	6960	3840
P. syringae pv. *lachrymans* USB327	1	NA	NA
P. syringae pv. *glycinea* NCPPB2752	4	870	960
P. tolaasii NCPPB 2192	2	NA	7680
Xanthomonas campestris pv. *phaseoli* NCPPB3035	0.0156	217.5	1920
Clavibacter michiganensis subsp. *michiganensis* DPP2	0.031	435	7680
C. michiganensis subsp. *sepedonicus* NCPPB2137	<0.0156	435	960
Curtobacterium flaccunfaciens pv. *flaccunfaciens* ICMP5370	0.125	217.5	NA
C. flaccunfaciens pv. *betae* NCPPB372	0.0625	435	NA

[a] MIQ, average quantity needed for the bacterial growth inhibition. The MIQ was calculated by considering the average densities of 0.87 and 0.96 g/mL of *C. sativum* and *F. vulgare* var. *vulgare* essential oils, respectively. NA, the deposition of 8 μL of essential oils on sterile blank disks did not lead to an inhibition zone.

for the human and animal pathogens (*25*−*28*), food spoilage bacteria (*29*, *30*), and some plant pathogenic bacteria and fungi (*7*, *10*−*12*, *31*). The results appear promising for a possible use of the above essential oils or their components as bactericides for the control of bacterial plant diseases. Of particular interest is the possibility to use these substances for seed treatments, which, when infected and/or contaminated, are the main source for the dissemination, also at a long distance, of a high number of phytopathogenic bacteria (*2*). So far, acid compounds (i.e., HCl, acetic acid, etc.), copper compounds, or chlorine derivatives, heat treatments as well as fermentation of fruits, from which seeds are extracted, have been used with a certain efficacy in seed sanitation from phytopathogenic bacteria (*2*). The availability of new active principles such as essential oils or their components is of great interest. Of course, other studies are necessary to evaluate the toxicity of the above substances toward seeds and/or plants and to set the appropriate formulations useful for the purpose.

Finally, the significant antibacterial activity of essential oils toward the bacterial pathogens of mushroom suggests the possibility to use the substances also on this crop. However, also in this case, further studies are necessary to evaluate the toxic effects of the essential oils on the mushroom production.

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