SUSCEPTIBILITY OF POSTHARVEST PATHOGENS TO ESSENTIAL OILS*

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Antimicrobial volatile substances from plants represent alternatives to synthetic pesticides and food preservatives. In this study, the compositions of some essential oils were determined by gas chromatography with mass spectrometry, and the inhibitory properties of the essential oils and their components against the bacterial postharvest pathogens *Pectobacterium carotovorum* subsp. *carotovorum* (CCM 1008), *Pseudomonas syringae* (CCM 7018), *Xanthomonas campestris* (CCM 22) were determined by the microdilution method. Essential oils from oregano, cinnamon, lemongrass, lavender, clove, rosemary, tea tree, eucalyptus, garlic, and ginger and their components cinnamaldehyde, eugenol, thymol, and carvacrol were used in the tests. The essential oil components exhibited strong antibacterial activity against all tested bacteria. The oregano and cinnamon essential oils were most effective. The rosemary, lavender, tea tree, eucalyptus, garlic, and ginger oils were not effective at the tested concentrations. In conclusion, certain essential oils, particularly their components, are highly effective and could be used for the control of postharvest bacterial pathogens.

essential oils, plant pathogens, antibacterial, *Pectobacterium, Pseudomonas, Xanthomonas*

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INTRODUCTION

Essential oils are aromatic oily liquids obtained from various plant parts and are often responsible for a plant's distinctive odour or taste. Consequently, essential oils play a prominent role as flavouring agents in the food industry and as fragrances in the perfume industry (Adlard, 2010). Essential oils are obtained from many plants that are traditionally used to enhance the taste or aroma of food and represent a complex mixture of natural substances. Essential oils possess antibacterial and antifungal activity and have been empirically used as antimicrobial agents (Burt, 2004; Bakkali et al., 2008). The antimicrobial activities of many essential oils have been reported previously (Alamshahi et al., 2010; Nezhad et al., 2012; Guerra et al., 2014; Mehrsorosh et al., 2014), and some studies have focused on the potential use of essential oils for the control of bacterial (Abanda-Nkpwatt et al., 2006; Tzortzakis, Economakis, 2007; Cho et al., 2010; Solórzano-Santos, Miranda-Novales, 2012; Badawy, Abdelgaleil, 2014) and fungal postharvest diseases (Aguiar et al., 2014; Badawy, Abdelgaleil, 2014; Castillo et al., 2014; Chen et al., 2014; Prakash et al., 2015; Frankova et al., 2016). Among plant pathogenic bacteria, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pseudomonas syringae,* and *Xanthomonas campestris*

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affect a wide range of plant species, including several economically important plants. *Pectobacterium carotovorum* subsp. *carotovorum* is a Gram-negative, rod-shaped, fermentative bacterium that causes bacterial soft rot and other diseases of many plant species, including potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), and cabbage (*Brassica campestris* L.) (Alamshahi et al., 2010; Zhao et al., 2013). *Pseudomonas syringae* is a Gram-negative polyphagous bacterium that usually survives as an epiphyte on host plants and becomes pathogenic under appropriate environmental conditions. This bacterium causes serious losses to stone fruits, in which it elicits a variety of symptoms, such as blossom blast, spur dieback, leaf necrosis, bark cankers, gummosis of woody tissues and bacterial spot (Huang, Lakshman, 2010; Kokoskova et al., 2011). Bacterial blight is usually caused by the Gram-negative rod-shaped bacterium *Xanthomonas campestris*. This seed-borne disease is characterized by necrotic lesions on leaves, stems, and/or fruits and affects cotton (*Gossypium herbaceum* L.), bean (*Phaseolus vulgaris* L.), and tomato (*Lycopersicon esculentum* Mill.) (Satish et al., 1999; Kotan et al., 2014).

In addition to decreased yield, plant pathogens can cause significant losses during storage (Kotan et al., 2014). Therefore, various treatments are applied to prevent postharvest pathogens from affecting the quality of stored products (Mahajan et al., 2014). Application of chemical pesticides can cause health hazards in animals and humans due to residual toxicity. Consequently, a number of synthetic pesticides have been banned (Satish et al., 1999). Moreover, many plant pathogenic bacteria have acquired resistance to synthetic pesticides (Kotan et al., 2014). Essential oils could be an alternative to synthetic pesticides (Božik et al., 2017; Frankova et al., 2016). In recent years, the search for alternative approaches to prolonging the shelf life of agriculture products has included extensive study of plant essential oils as potential tools for postharvest treatment and food preservation (Tzortzakis, 2007; Peretto et al., 2014; Sivakumar, Bautista-Baños, 2014). Thus, in this study, we evaluated the antibacterial activities of selected essential oils and their constituents against three important plant pathogens: *Pectobacterium carotovorum* subsp. *carotovorum*, *Pseudomonas syringae,* and *Xanthomonas campestris*.

MATERIAL AND METHODS

Bacterial strains

In this study, we used three potentially phytopathogenic Gram-negative bacterial strains (*Pectobacterium carotovorum* subsp. *carotovorum* (CCM 1008), *Pseudomonas syringae* (CCM 7018), and *Xanthomonas campestris* (CCM 22)) obtained from the Czech Collection of Microorganisms (Brno, CZ) and maintained in tryptone soya broth (Oxoid Ltd., Basingstoke, UK) at 25°C.

Essential oils and their components

Essential oils from oregano (*Origanum vulgare*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), lemongrass (*Cymbopogon citratus*), lavender (*Lavandula officinalis*), rosemary (*Rosmarinus officinalis*), tea tree (*Melaleuca alternifolia*), eucalyptus (*Eucalyptus globulus*), garlic (*Allium sativum*), and ginger (*Zingiber officinale*) were purchased from Biomedica (Prague, Czech Republic) and Sigma-Aldrich (St. Louis, USA) and stored at 4°C in airtight sealed glass bottles. Cinnamaldehyde, eugenol, thymol, and carvacrol used in tests were purchased from Sigma-Aldrich.

Analysis of essential oils

Gas chromatography with mass spectrometry (GC-MS) was used to identify the constituents of the essential oils. The essential oils were diluted in hexane to a final concentration of 1 μl ml^{-1}. The GC-MS analyses were performed using an Agilent 7890A GC coupled to an Agilent MSD5975C MS detector (Agilent Technologies, Palo Alto, USA) with an HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness). A 1-μl aliquot of the sample was injected in split mode 1 : 12, with an injector temperature of 250°C and electron ionization energy of 70 eV. Analyses were performed in SCAN mode with a mass range of 40–400 m z^{-1}. The oven temperature started at 60°C and was programmed to 231°C at a rate of 3°C min^{-1}; the final temperature was then held for 10 min (Kloucek et al., 2012). The identification of constituents was based on a comparison of their mass spectra and relative retention indices with the National Institute of Standards and Technology Library (NIST, USA), as well as authentic standards and the literature (Adams, 2007). The standards from Sigma-Aldrich (St. Louis, USA) and their retention indexes (RI) are presented in Table 1.

Antimicrobial assays

Before the antimicrobial assays, stock cultures of the bacterial strains were grown in tryptone soya broth (Oxoid, Ltd., Basingstoke, UK) at 25°C for 24 h for *Pectobacterium* or 72 h for *Pseudomonas* and *Xanthomonas*. An inoculum was then created by dilution in the same medium to a final cell concentration of 10^6 CFU ml^{-1}, which was confirmed by measuring the cell density in McFarland units (densitometer McFarland type DEN-1B; Biosan, Riga, Latvia). A modification of the EUCAST (2003) microdilution method was

Table 1. Retention indexes (RI) of used standards

RI	standard	RI	standard	RI	standard
921	anisole	1089	(+)-fenchone	1223	(-)-menthone
937	α-pinene	1102	linalool	1234	β-citronellol
952	camphene	1112	rose oxide	1244	citral
963	benzaldehyde	1146	camphor	1247	(-)-carvone
979	β-pinene	1149	isopulegol	1259	geraniol
993	β-myrcene	1158	(+/-)-citronellal	1271	*trans*-cinnamaldehyde
999	butylisothiocyanate	1169	borneol	1287	(-)-bornyl acetate
1019	α-terpinene	1176	(+)-menthol	1296	thymol
1028	p-cymene	1181	4-terpineol	1306	carvacrol
1032	limonene	1198	D-dihydrocarvone	1361	eugenol
1034	eucalyptol	1199	estragole	1387	geranyl acetate
1063	γ-terpinene	1202	2-decanol	1420	β-caryophyllene
				1448	citronellyl propionate

used for antimicrobial testing. A two-fold serial dilution of an essential oil or component ranging from 1024 to 64 mg l^{-1} was prepared from a stock solution in tryptone soy broth with 1% Tween 80. Then 190 µl were pipetted to 96-well microtitration plates, followed by the addition of 10 µl of inocula. The microtitration plates were incubated at 25°C for 72 h. After incubation, the minimum inhibitory concentrations (MICs) were recorded. MICs were expressed as the lowest concentration at which a substance absolutely inhibited visible growth of the bacterium. Each plate contained two negative and two positive controls. For each essential oil or component, the microdilution assay was performed in triplicate, and the resulting median MICs were recorded.

RESULTS

Compositions of essential oils

The compositions of the essential oils were analyzed by GC-MS (Kloucek et al., 2012). The percentage compositions and modes of identification of the oil components are listed in Table 2. The chromatographic analyses resulted in the identification of 108 components representing 86.7–99.67% of the oils. The major components (Table 2) of the essential oils were eugenol in clove (82%) and cinnamon oil (72%), eucalyptol in eucalyptus oil (82%) and rosemary oil (44%), diallyl disulphide (39%) and diallyl trisulfide (20%) in garlic oil, linalyl acetate (32%) and linalool (30%) in lavender oil, α- and β-citral (70% together) in lemongrass oil, carvacrol in oregano oil (67%), 4-terpineol (37%) and γ-terpinene (19%) in tea tree oil and zingiberene in ginger (32%).

MIC determination

The MICs of the 10 plant essential oils and 4 essential oil components against *Pectobacterium carotovorum* subsp. *carotovorum*, *Pseudomonas syringae*, *Xanthomonas campestris* obtained by the microdilution method are shown in Table 3. The most effective essential oils were those from oregano (MIC 256, 256, and 64 mg l^{-1}, respectively) and cinnamon (MIC 128, 256, and 256 mg l^{-1}). The MICs of cinnamaldehyde (MIC 128, 128, and 64 mg l^{-1}) and eugenol (MIC 256, 512, and 128 mg l^{-1}) were lower than those of the essential oils from cinnamon (MIC 128, 256, and 256 mg l^{-1}) and clove (MIC 1024, 1024, and 512 mg l^{-1}), respectively. By contrast, thymol (MIC 512, 1024, and 512 mg l^{-1}) and carvacrol (MIC 512, 1024, and 1024 mg l^{-1}) were less effective than oregano oil. The essential oils of lemongrass, lavender, rosemary, tea tree, and eucalyptus were active against *Xanthomonas campestris* only (MICs ranging from 256 to 1024 mg l^{-1}), with the exception of lavender, which also showed weak activity against *Pectobacterium carotovorum*. The garlic and ginger oils were not active against any microorganism tested. *Xanthomonas campestris* was the most susceptible microorganism, with sensitivity to the majority of tested substances, whereas *Pseudomonas syringae* was the least susceptible microorganism.

DISCUSSION

Our results for the analysis of the compositions of essential oils are consistent with previously published data (Cavanagh, Wilkinson, 2002; Pawar, Thaker, 2006; Horváth et al., 2009; Teixeira et al., 2013; Raut, Karuppayil, 2014). The

Table 2. Essential oils composition determined by gas chromatography–mass spectrometry - Part 1

RI[b]		Compound	Cinnamon	Clove	Eucalyptus	Garlic	Lavender	Lemongrass	Oregano	Rosemary	Tea tree	Ginger
918		methyl allyl disulfide				3.39						
937	a	α-pinene	1.04		6.89		0.24	0.37	0.41	10.98	2.79	2.93
952	a	camphene	0.33		0.03		0.14	2.11	0.17	5.13		9.95
963	a	benzaldehyde	0.18									
972		dimethyl trisulfide				0.04						
976		β-thujene					0.04			0.06	0.14	0.06
979	a	β-pinene	0.21		0.41		0.03		0.80	7.61	0.72	0.22
981		1-octen-3-ol					0.23		0.22			
988		3-octanone					1.77		0.14			
988		sulcatone		0.02				1.84				0.50
992		β-myrcene	0.07		0.35		0.58		0.52	0.72	0.66	0.56
998		3-octanol					0.44	0.03	0.19			
999		myrac aldehyde								0.04		
1004		octanal						0.08				
1005		α-phellandrene	0.80		0.31		0.03		0.13	0.04	0.46	0.30
1012		3-carene	0.08				0.14		0.02	0.08		0.02
1016		acetic acid hexyl ester					0.61					
1019	a	α-terpinene	0.12		0.05		0.03		0.42		9.17	
1024		*o*-cymene	0.02				0.04					
1027	a	*p*-cymene	1.22		5.47		0.29		10.32	3.28	5.97	0.07
1031	a	D-limonene	0.71				1.04	0.41	0.67		1.72	
1032		β-phellandrene										8.99
1034	a	eucalyptol	0.12		82.55		0.65		0.99	43.86	3.03	2.37
1042		*trans*-β-ocimene			0.07		3.14	0.14				
1052		*cis*-β-ocimene	0.02		0.03		2.01	0.12				
1062		γ-terpinene	0.03		2.00		0.11		2.56		19.04	0.02
1069		*trans*-4-thujanol							0.06	0.04		
1070		α-terpinene					0.06					
1075		1,2-epoxylinalool							0.06			
1075		*cis*-linaloloxide					0.16					
1075		4-nonanone						1.24				
1082		diallyl disulphide				38.70						
1089		(+)-4-carene										0.15
1089		terpinolene	0.09		0.16		0.20	0.09	0.16		3.43	
1093		2-nonanone				0.08						0.03
1096		2-nonanol										0.04
1098		ethyltetramethyl-cyclopentadiene						0.08				
1098		durenol										0.11
1100	a	linalool	1.87		0.05		29.89	1.32	1.45	0.51	0.04	0.15
1115		1-octen-3-yl-acetate					1.32					
1132		*trans*-alloocimene					0.15					
1139		methyl allyl trisulfide				2.48						
1146	a	camphor					0.21		0.69	13.19		0.08

Table 2. Essential oils composition determined by gas chromatography–mass spectrometry - Part 2

RI[b]		Compound	Cinnamon	Clove	Eucalyptus	Garlic	Lavender	Lemongrass	Oregano	Rosemary	Tea tree	Ginger
1149	[a]	isopulegol										0.04
1156	[a]	(+/-)-citronellal						0.21				0.01
1164		benzenepropanal	0.02									
1167	[a]	borneol					0.58		1.03	3.86		0.75
1170		lavandulol					0.91					
1178	[a]	4-terpineol	0.10		0.34		4.92		0.96	0.39	36.87	
1185		3-vinyl-1,2-dithia cyclohex-4-ene				0.58						
1186		*p*-cymenene	0.04						0.04			
1190		α-terpineol	0.24		0.84		1.30	0.27	0.56	2.12	2.89	0.44
1194		methyl salicylate		0.12								
1206		decanal										0.12
1207		piperitol									0.09	
1209		3-vinyl-1,2-dithia cyclohex-5-ene				1.84						
1221		fenchyl acetate							0.04			
1230		*cis*-geraniol					0.18					0.05
1240		2-methyl-3-phenyl-propanal							0.18			
1245	[a]	β-citral						31.44				0.05
1257	[a]	geraniol						4.55				
1260		linalyl acetate					31.73					
1269		*trans*-cinnam-aldehyde	1.35									
1274		geranial (α-citral)					0.04	39.96				3.08
1285		(-)-bornyl acetate	1.33				0.16		0.14	1.66		
1292		lavandulyl acetate					3.85					
1293		2-undecanone										0.15
1296	[a]	thymol							5.14	0.11		
1297		diallyl trisulfide				19.90						
1305	[a]	carvacrol							67.14			
1350		cubebene		0.12						0.08	0.06	
1364	[a]	eugenol	76.85	81.74		1.57						
1365		(+)-cycloiso-sativene					0.35	0.28				0.32
1376		α-cubebene	0.68	0.42					0.10	0.19	0.15	0.61
1377		allyl methyl tetrasulfide				0.75						
1385		geranyl acetete					0.66	4.15				0.29
1392		β-elemene						0.18			0.05	0.90
1396		vanilin	0.04	0.09								
1405		eugenol methyl ether	0.04	0.06								
1408		α-gurjunene									0.46	0.05
1419	[a]	β-caryophyllene	2.97	12.25			4.85	1.99	1.09	3.10	0.46	0.09
1427		γ-maaliene									0.09	
1432		β-gurjunene									0.02	0.03
1434		γ-elemene										0.45
1439		(-)-spathulenol	0.06		0.11				0.04		1.52	0.90

Table 2. Essential oils composition determined by gas chromatography–mass spectrometry - Part 3

RI[b]		Compound	Cinnamon	Clove	Eucalyptus	Garlic	Lavender	Lemongrass	Oregano	Rosemary	Tea tree	Ginger
1443		β-farnesene									0.18	
1446		cinnamyl acetate	1.38									
1450		humulen-(v1)		0.03					0.03			
1453		α-caryophyllene	0.53	1.49			0.12	0.22	0.35	0.29	0.13	0.07
1459		*cis*-β-farnesene					3.30					
1460		(+)-spathulenol							0.03		0.67	1.08
1473		γ-gurjunene									0.56	
1477		β-amorphene							0.09			
1480		D-germacrene						0.04				1.51
1484		α-curcumene										10.02
1492		γ-maaliene										1.30
1494		(+)-ledene	0.09						0.07		1.56	
1497		zingiberene										32.48
1499		β-cadinene						0.15	0.07		0.18	0.58
1509		β-bisabolene					0.05		0.11			7.84
1513		α-muurolene					0.11	2.17	0.06		0.02	
1523		δ-cadinene	0.14	0.31				0.49	0.27	0.04	2.02	
1529		eugenol acetate	2.21	0.05								
1538		diallyl tetra-sulphide				8.26						
1573		caryophyllene oxide	0.48	2.54			0.41	0.61	1.35	0.96		
1603		α-bergamotene										0.29
1763		benzyl benzoate	3.87									
1810		4-thiazolidinone				6.51						
2006		sulfuric compound				2.58						
total			99.31	99.23	99.67	86.68	97.06	94.57	98.88	98.31	95.13	90.05

[a] identification confirmed by co-injection of authentic standard

[b] retention indexes (RI): identification based on Kovats retention indices (HP-5MS capillary column) and mass spectra

antimicrobial activity of essential oils as well as the effectiveness of their active components have also been extensively investigated (Burt, 2004; Kotan et al., 2014; Yong et al., 2015). The bioactivity of essential oils is generally attributed to phenolic compounds (phenols), which are soluble in the lipid layer of the membrane and alter membrane fluidity (Horváth et al., 2009; Bevilacqua et al., 2010). The results of different studies are difficult to compare, presumably because of the use of different test methods, bacterial strains, and sources of antimicrobial samples. The antibacterial effects of thyme oil, carvacrol, and thymol on *Pectobacterium* were assessed by measuring inhibition zones using the agar diffusion method (Karami-Osboo et al., 2010). The compositions of some essential oils and their MICs against plant pathogenic bacteria obtained by the agar dilution method were previously reported. The oil of *Origanum vulgare* strongly inhibited the growth of *Pectobacterium* with an MIC of 400 mg l^{-1} (Badawy, Abdelgaleil, 2014). Kokoskova et al. (2011) compared antimicrobial activity of streptomycin (0.02%) and essential oils from *Origanum compactum* (main components: carvacrol 36.2%, P-cymene 22.3%, thymol 18.6%), *Origanum vulgare* (thymol 28.5%, carvacrol 19.5%), and *Thymus vulgaris* (P-cymene 16.3%, geraniol 8.3%, carvacrol 7.9%, thymol 6.8%). These essential oils exhibited higher antibacterial activity than streptomycin when tested against the plant pathogens *Pseudomonas syringae* and *Erwinia amylovora* by the disc diffusion method (Kokoskova et al., 2011). In the present study, oregano and cinnamon showed the greatest effects against all bacteria tested, and the oils were more effective than their major constituents. Typically, essential oils have stronger antimicrobial effects than their components alone due to synergic effects of the components (Calo et al., 2015; Elshafie et al., 2015). In our study, eugenol, the main component of clove and cinnamon essential oil, had stronger antibacterial activity than the essential oil. Eugenol is the strongest antimicrobial compound in clove oil but it represents only 81.74% of whole oil. Cinnamon oil has similar content of eugenol (76.85%), but it also contains 3.7% of benzyl benzoate which is a strong antimicrobial substance. Results from this study confirmed that oregano and cinnamon essential oils and their compounds (cinnamaldehyde, thymol, carvacrol) have high antibacterial potential and can be effectively used. The use of essential oils as pesticides is safer than that of chemicals and could become good alternative for them (Karami-Osboo et al., 2010; Zarubova et al., 2015).

Table 3. Minimum inhibitory concentrations (MICs) of essential oils against plant pathogens

Essential oil	MIC (mg l^{-1})		
	PCC	PS	XC
Oregano	256	256	64
Cinnamon	128	256	256
Lemon grass	> 1024	> 1024	512
Lavender	1024	> 1024	256
Clove	1024	1024	512
Rosemary	> 1024	> 1024	1024
Tea tree	> 1024	> 1024	1024
Eucalyptus	> 1024	> 1024	1024
Garlic	> 1024	> 1024	> 1024
Ginger	> 1024	> 1024	> 1024
Essential oil compound			
Cinnamaldehyde	128	128	64
Eugenol	256	512	128
Thymol	512	1024	512
Carvacrol	512	1024	1024

PCC = *Pectobacterium carotovorum* subsp. *carotovorum* (CCM 1008), PS = *Pseudomonas syringae* (CCM 7018), XC = *Xanthomonas campestris* (CCM 22)

CONCLUSION

In the present study, we evaluated and compared the antibacterial activities of 10 plant essential oils and their compounds against three postharvest plant pathogens. In conclusion, our results showed good antibacterial potential of the oils against the bacterial pathogens tested, however, further research is needed in order to evaluate the efficacy of these oils in model products, to identify suitable products, and to adjust the application method.

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