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In vivo antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit

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ABSTRACT

Fresh fruits of several plants are susceptible to infection by several pathogenic fungi after harvest. Some synthetic fungicides are known to be highly effective in their control on various vegetables and fruits. In the present study the potential fungicidal activity of the essential oils obtained by thyme (*Thymus vulgaris*) and vervain (*Verbena officinalis*), respectively, against *Monilinia laxa*, *Monilinia fructigena*, and *Monilinia fructicola* was tested at various concentrations *in vivo*. The oil of thyme was mainly composed by *o*-cymene (56.2%), while the main components of the oil of vervain were citral (44.5%) and isobornyl formate (45.4%). The higher concentrations of both studied EOs from vervain (1000 ppm) and thyme (500 ppm) significantly reduced the brown rot lesion diameter. The lower concentrations of vervain (500 ppm) and thyme (250 ppm) EOs resulted in low effectiveness. This research revealed the potential fungicidal role *in vivo* of the essential oils on peach fruits postharvest. Moreover, the application of essential oils could be combined with other innovative postharvest treatments such as biocontrol agents.

1. Introduction

Fresh fruits of several plants are susceptible to infection by several pathogenic fungi after harvest. Some synthetic fungicides are known to be highly effective in their control on various vegetables and fruits. However, the use of synthetic fungicides is limited by the emergence of resistant fungus strains and, in some cases, their use is prohibited by law in postharvest phase. The growing of public concern over the health and environmental hazards associated with the increased levels of pesticide use in fruit orchards and the lack of continued renewed approval of some of the most effective active molecules have lead to develop alternative, safe and natural methods postharvest control (Lopez-Reyes et al., 2013). Recently, there has been a great interest in using essential oils (EOs) and plant extracts as possible natural substitutes for conventional synthetic pesticides. This has been mainly due to the concern over ecosystem pollution and pesticide resistance in pests and fungal pathogens (Holmes and Eckert, 1999; Camele et al., 2010).

EOs are volatile compounds produced in many plant species. These oils are thought to play a role in plant defense mechanisms against phytopathogenic microorganisms (Liu and Chu, 2002). EOs have been reported to control *Monilinia laxa* (Aderh. & Ruhland) Honey in stone fruit (Neiri et al., 2007) postharvest diseases in tomato, citrus, molds, food-borne, various bacteria (Banihashemi and Abivardi, 2011) and weeds (Macias et al., 2007). EO from *Thymus capitatus* (L.) Hoffm. & Link displayed antifungal activity on stored foods and inhibited the growth of both *Botrytis cinerea* (de Bary) Whetzel and *Monilinia fructicola* (G. Winter) Honey (Tsao and Zhou, 2000). The potential use of EOs by spraying or dipping techniques to control postharvest decay has been studied in vegetables, fruits and cut flowers (Dixit et al., 1995).

In vivo antifungal activity of some EOs derived from some Mediterranean plants such as *Thymus vulgaris* L., *Verbena officinalis* L. and *Origanum vulgare* (Link) Ietswaart, has been proved against some postharvest fungal pathogens such as *B. cinerea*, *Penicillium italicum* Wehmer, *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.) Leonian and *Rhizopus stolonifer* (Ehrenb. Fr.) Vuill. In particular, the most effective EO was extracted from *T. vulgaris*, which controlled at 2000 ppm dose, the fruit rot caused by *B. cinerea*, *P. citrophthora* and *R. stolonifer* but was ineffective against *P. italicum*. The EOs extracted from *V. officinalis* inhibited infection caused by *B. cinerea*, *P. citrophthora* and O. *vulgare* oil was effective only against *P. citrophthora* (Camele et al., 2010). *Monilinia* spp. are responsible for brown rots (De Cal and Melgarejo, 1999) serious postharvest

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stone fruits diseases which cause significant losses in most temperate regions of the world. In particular, *M. fructicola* is included in the EPPO (European and Mediterranean Plant Protection Organization) A2 list of quarantine pest. *M. fructicola*, regularly present in Asia, North America and Australia, has been recently detected in Italy (Pellegrino et al., 2009) and other European countries, where it is rapidly replacing *M. laxa* as the main agent of brown rot on stone fruits (Lopez-Reyes et al., 2013). In this study, the potential fungicidal activity of two EOs, previously selected in tests *in vitro* by Camele et al. (2010), was investigated *in vivo* against three

Table 1
Percent compositions of thyme and vervain essential oils.

N.	Compound	Ki[a]	Ki[b]	*T. vulgaris* (%)	*V. officinalis* (%)	Identification[c]
1	α-Thujene	930	1035	t[d]	-[e]	1, 2
2	α-Pinene	938	1032	2.5 ± 0.1	0.2 ± 0.0	1, 2, 3
3	(-)-Camphene	953	1076	1.0 ± 0.1	-	1, 2, 3
4	Sabinene	973	1132	t	0.5 ± 0.0	1, 2, 3
5	Hepten-3-one	975		-	0.2 ± 0.1	1, 2
6	β-Pinene	978	1118	-	t	1, 2, 3
7	Verbenene	982	1131	t	-	1, 2
8	Myrcene	993	1174	0.1 ± 0.0	-	1, 2, 3
9	α-Phellandrene	995	1176	t	-	1, 2, 3
10	α-Terpinene	1012	1188	0.1 ± 0.0	t	1, 2, 3
11	*o*-Cymene	1020	1187	56.2 ± 0.2	0.1 ± 0.0	1, 2, 3
12	*p*-Cymene	1024	1280	0.1 ± 0.0	-	1, 2, 3
13	β-Phellandrene	1029	1218	0.2 ± 0.1	0.7 ± 0.2	1, 2, 3
14	Limonene	1030	1203	0.6 ± 0.0	2.3 ± 0.9	1, 2, 3
15	1,8-Cineole	1034	1213	t	0.4 ± 0.1	1, 2
16	(Z)-β-Ocimene	1038	1246	t	t	1, 2, 3
17	(E)-β-Ocimene	1049	1280	t	0.3 ± 0.1	1, 2, 3
18	γ-Terpinene	1057	1255	0.4 ± 0.0	0.1 ± 0.0	1, 2, 3
19	Terpinolene	1086	1265	0.7 ± 0.1	t	1, 2
20	Linalol	1097	1553	0.4 ± 0.1	0.1 ± 0.0	1, 2, 3
21	*cis*-Thujone	1105	1430	t	-	1, 2, 3
22	*trans*-Pinocarveol	1138	1654	t	t	1, 2
23	(-)-Citronellal	1143	1491	0.5 ± 0.1	-	1, 2, 3
24	*iso*-Borneol	1144	1633	0.1 ± 0.0	-	1, 2, 3
25	Camphor	1145	1532	t	-	1, 2, 3
26	*iso*-Pinocamphone	1153	1566	t	0.2 ± 0.0	1, 2
27	*trans*-Pinocamphone	1159	1160	t	t	1, 2
28	*iso*-Menthone	1163	1503	0.1 ± 0.0	-	1, 2, 3
29	Pinocarvone	1165	1587	t	t	1, 2
30	Borneol	1167	1719	0.2 ± 0.0	0.1 ± 0.0	1, 2, 3
31	Terpinen-4-ol	1176	1611	t	0.2 ± 0.0	1, 2, 3
32	dihydro-Carveol	1177	1755	0.2 ± 0.0	-	1, 2
33	*p*-Cymen-8-ol	1185	1864	t	t	1, 2
34	α-Terpineol	1189	1706	0.3 ± 0.0	0.3 ± 0.1	1, 2, 3
35	Myrtenal	1193	1648	0.3 ± n0.0	-	1, 2
36	Myrtenol	1196	1804	0.3 ± 0.0	-	1, 2
37	Isobornyl formate	1228	1596	-	45.4 ± 0.9	1, 2
38	*cis*-Anethole	1262	1780	-	0.2 ± 0.0	1, 2
39	(E)-Citral	1270	1727	-	44.5 ± 0.9	1, 2, 3
40	Isobornyl acetate	1277		t	t	1, 2
41	Bornyl acetate	1284	1591	t	t	1, 2
42	Thymol	1290	2198	8.7 ± 0.9	-	1, 2, 3
43	Carvacrol	1297	2239	24.4 ± 0.9	-	1, 2, 3
44	Methyl eugenol	1369	2023	-	t	1, 2
45	α-Copaene	1377	1497	t	0.2 ± 0.1	1, 2
46	Isoledene	1382	1367	t	0.1 ± 0.0	1, 2
47	β-Elemene	1387	1600	t	0.2 ± 0.1	1, 2
48	Longifolene	1411	1576	t	t	1, 2
49	β-Caryophyllene	1418	1612	0.1 ± 0.0	0.1 ± 0.1	1, 2
50	β-Cedrene	1424	1638	-	0.4 ± 0.1	1, 2
51	Aromadendrene	1437	1628	t	-	1, 2
52	α-Humulene	1455	1689	t	0.2 ± 0.0	1, 2
53	allo-Aromadendrene	1463	1661	t	0.1 ± 0.0	1, 2
54	γ-Gurjunene	1473	1687	t	t	1, 2
55	Bicyclogermacrene	1491	1756	-	0.1 ± 0.0	1, 2
56	*cis*-Muurola-4(14),5-diene	1510	1675	t	0.2 ± 0.1	1, 2
57	α-7-*epi*-Selinene	1518	1740	t	0.2 ± 0.1	1, 2
	Total			97.5	97.6	
	Monoterpenes hydrocarbons			61.9	4.2	
	Oxygenated monoterpenes			35.5	91.2	
	Sesquiterpenes			0.1	1.8	
	Oxygenated sesquiterpenes			-	-	
	Other compunds			-	0.4	

[a] Kovats retention index on HP-5 MS column.
[b] Kovats retention index on HP Innowax column.
[c] 1 = Kovats retention index, 2 = mass spectrum, 3 = co-injection with authentic compound.
[d] t = trace, less than 0.05%.
[e] - = not detected.