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Cupressus sempervirens essential oils and their major compounds successfully control postharvest grey mould disease of tomato

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A R T I C L E I N F O

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A B S T R A C T

Medicinal plants generally produce many secondary metabolites which constitute an important source of many bioactive molecules. Among them *Cupressus sempervirens* is recognized by its richness in essential oil with antimicrobial properties. The aim of this study is to use *C. sempervirens* essential oil or its bioactive components as an alternative to synthetic fungicides currently used to control *Botrytis cinerea*. Essential oils of *C. sempervirens* were extracted at vegetative, flowering and fructification stages and a total of 54 compounds were identified by chromatography-mass spectrometry. Essential oils composition varied with the phenological stage and the main chemical classes were sesquiterpene hydrocarbons (59.59%–64.5%) with the most representative compounds being germacrene D (18.38%–24.82%) and those of the monoterpene hydrocarbons class (16.63%–26.5%) with α-pinene as the most representative compound (14.75%–22.92%). The *in vitro* antifungal tests against *B. cinerea* showed that the three studied essential oils inhibit mycelia growth with the highest activity observed at flowering stage. The antifungal activity of some pure compounds (α-pinene, α-cedrol and β-caryophyllene) alone or combined according to their proportions in the natural essential oil showed that α-pinene combined with β-caryophyllene provided the highest antifungal activity at a concentration as low as 0.12 mg/mL as compared to that of the chemical fungicide used as a positive control. Microscopic observation showed that essential oil at flowering stage induced swelling and crumbling of *B. cinerea* conidia. The pulverization of *C. sempervirens* essential oils on tomato fruits at 1 mg/mL inhibited 54% of *B. cinerea* infection which constitute a promising safe product for the biocontrol of the post-harvest disease *Botrytis cinerea* during storage and transport of tomato.

1. Introduction

Tomato (*Lycopersicon esculentum Mill*) is worldwide cultivated, occupying about 8 million hectares and with production of 217 million of tons (Fiume and Fiume, 2006). Tomato is the second most consumed vegetable in the world after potato. In addition, tomatoes are low in calories (20 calories per average size fruit) and they are an excellent source of iron and vitamins A and C. They also contain carotenoids, such as lycopene with antioxidant properties, and small amounts of the B complex vitamins thiamin, niavin, and riboflavin (Fanascas et al., 2006). However, tomato crops can be exposed to different dangerous fungus, among them *Botrytis cinerea* that is a necrophic plant pathogenic fungus that infects about 235 host species. In addition, grey mould, caused by *Botrytis cinerea* reduces the productivity of greenhouse grown tomatoes in many regions of the world through its infection on leaves, stems and fruits (Wang et al., 2016). Infestation is stimulated by high humidity, particularly if free moisture is present on the plant surface at low temperatures (Williamson et al., 2007). The control of grey mould is generally achieved by the use of synthetic fungicides (Elad et al., 1995), which have many negative consequences. In fact, the use of synthetic fungicides leads to the emergence of resistant fungal strains and in some circumstances causes human toxicity which

*Abbreviations:*EOs, essential oils
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Table 1
Chemical composition of *Cupressus sempervirens* essential oils at three phenological stages.

Compounds	% of total aroma		
	Vegetative stage	Flowering stage	Fructification stage
α-Thujene	0.15^{b} ± 0.01	0.36^{a} ± 0.03	nd
α-Pinene	**14.75^{b} ± 0.75**	**22.92^{a} ± 2.94**	**16.19^{b} ± 0.48**
Camphene	nd	0.33^{a} ± 00.00	nd
β-Pinene	0.17^{a} ± 0.18	nd	nd
Myrcene	0.15^{a} ± 0.37	nd	nd
δ-3-Carene	nd	1.30^{b} ± 0.08	5.75^{a} ± 0.04
Limonene	0.97^{b} ± 0.07	1.30^{a} ± 0.04	1.35^{a} ± 0.00
p-Cimene	0.14^{a} ± 0.13	nd	nd
δ-Terpinene	0.30^{b} ± 0.04	0.32^{b} ± 0.02	0.40^{b} ± 0.00
α-Terpinolene	1.02^{b} ± 0.07	0.91^{c} ± 0.03	1.69^{a} ± 0.02
trans-Pinocarveol	0.37^{a} ± 0.14	0.39^{a} ± 0.02	nd
α-Phellandrene-8-ol	0.67^{a} ± 0.18	0.38^{b} ± 0.01	nd
Terpinene-4-ol	0.95^{a} ± 0.07	0.61^{b} ± 0.15	0.32^{c} ± 0.00
α-Terpineol	0.32^{b} ± 0.04	0.16^{c} ± 0.01	0.93^{a} ± 0.00
Carvacrolmethylether	0.84^{a} ± 0.05	0.87^{a} ± 0.00	0.67^{b} ± 0.00
Borneol.acetate	0.54^{a} ± 0.02	0.43^{b} ± 0.01	0.38^{c} ± 0.00
m-Mentha-1.8-diene	nd	0.32^{a} ± 0.01	nd
α-Terpinylacetate	0.42^{a} ± 0.42	nd	0.35^{a} ± 0.00
Alloaromadendrene	nd	0.48^{b} ± 0.01	1.00^{a} ± 0.05
(+)-4-Carene	4.96^{a} ± 1.32	3.60^{a} ± 0.09	6.63^{a} ± 0.03
Ylangene	nd	0.25^{a} ± 0.01	nd
α-Copaene	0.72^{b} ± 0.03	0.82^{a} ± 0.02	0.67^{c} ± 0.0
β-Bourbonene	nd	0.16^{a} ± 0.01	nd
Cis-muurola-3.5-diene	0.45^{b} ± 0.00	0.51^{a} ± 0.01	0.43^{c} ± 0.00
α-Farnesene	nd	0.21^{a} ± 0.06	nd
(+)-.β.-Funebrene	2.47^{b} ± 0.11	3.06^{a} ± 0.08	2.63^{b} ± 0.02
β-Caryophyllene	5.29^{a} ± 0.21	5.29^{a} ± 0.19	3.39^{b} ± 0.04
β-Ylangene	0.54^{a} ± 0.15	nd	nd
β-Cubebene	nd	0.58^{a} ± 0.15	0.43^{a} ± 0.21
δ-Cadinene	1.73^{b} ± 0.04	0.77^{c} ± 0.02	2.75^{a} ± 0.03
α-Caryophyllene	4.46^{a} ± 0.24	4.21^{a} ± 0.08	2.36^{b} ± 0.03
epi-Bicyclosesquiphellandrene	4.03^{b} ± 0.12	2.44^{c} ± 0.09	6.83^{a} ± 0.10
α-Amorphene	nd	2.86^{a} ± 0.07	1.47^{b} ± 0.09
δ-Muurolene	2.95^{a} ± 1.07	nd	nd
Germacrene D	**18.38^{c} ± 0.18**	**20.66^{b} ± 0.38**	**24.82^{a} ± 0.14**
Longipinene	1.31^{a} ± 0.33	1.18^{a} ± 0.04	nd
α-Ylangene	0.41^{ab} ± 0.82	nd	0.87^{a} ± 0.05
α-Muurolene	2.15^{ab} ± 0.67	1.72^{b} ± 0.02	2.57^{a} ± 0.04
δ-Cadinene	2.86^{a} ± 0.11	nd	nd
(+)-β-Gurjunene	nd	3.29^{a} ± 0.06	2.15^{b} ± 0.03
β-Cadinene	7.39^{a} ± 0.33	7.06^{a} ± 0.16	4.84^{b} ± 0.07
β-Muurolene	0.32^{b} ± 0.00	0.29^{c} ± 0.00	0.43^{a} ± 0.01
α-Cadinene	nd	nd	0.24^{a} ± 0.00
Ledeneoxide-(II)	0.35^{a} ± 0.02	0.16^{b} ± 0.08	nd
Cadinol	nd	nd	0.25^{a} ± 0.01
Caryophylleneoxide	0.46^{a} ± 0.02	0.32^{b} ± 0.00	nd
Aromadendreneoxide	0.31^{a} ± 0.03	0.25^{a} ± 0.11	nd
α-Cedrol	0.81^{a} ± 0.33	5.99^{b} ± 0.07	5.61^{c} ± 0.09
Drimenol	0.37^{a} ± 0.06	0.40^{a} ± 0.00	nd
Cubenol	0.18^{ab} ± 0.22	0.27^{a} ± 0.00	nd
Muurolol	0.39^{a} ± 0.01	0.22^{b} ± 0.00	nd
α-Cadinol	1.23^{a} ± 0.31	0.40^{c} ± 0.00	0.65^{b} ± 0.01
Sclareol	0.30^{a} ± 0.64	nd	nd
Unknown	1.02 ± 0.01	0.77 ± 0.00	0.46 ± 0.00
Chemical Classes			
Monoterpenehydrocarbons	16.63^{c} ± 0.17	26.53^{a} ± 0.34	23.69^{b} ± 0.05
Oxygenated monoterpenes	5.13^{a} ± 0.06	4.79^{b} ± 0.03	4.34^{b} ± 0.01
Sesquiterpenehydrocarbons	60.42^{b} ± 0.21	59.59^{b} ± 0.10	64.51^{a} ± 0.16
Oxygenatedsesquiterpenes	4.87^{c} ± 0.02	8.06^{a} ± 0.01	6.51^{b} ± 0.01
Others	1.43^{a} ± 0.01	1.02^{a} ± 0.01	1.1^{a} ± 0.00

Components are listed in order of elution in polar column (HP-Innowax); nd: not detected. Means in the same column and followed by the same letter are not significantly different at ($P \leq 0.05$) using Duncan's multiple range test.
Bold values represent the percentages of the main volatile compounds.

restricts their use in postharvest disease control (Elshafie et al., 2015). Nowadays, there is a growing public concern over the health and environmental hazards associated with the use of pesticides that leads to the development of safe alternatives and natural methods in postharvest diseases control (Hukkanen et al., 2007). Among the various alternatives, natural plant products including essential oils (EOs) are biodegradable, cheaper, and environmentally safe and thereby, they are attracting the attention of scientists, farmers and industrialists worldwide (Abd-Alla and Haggag, 2013; Zhu et al., 2016). These secondary metabolites are volatile compounds produced in many plant species in which they are thought to play a role in defense mechanisms against phytopathogens (Liu and Chu, 2002).