Efficacy of plant essential oils on the control of postharvest rots of stone fruits.

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Efficacy of plant essential oils on postharvest control of rot caused by fungi on four cultivars of apples *in vivo*

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Running head: "Essential oils applied on postharvest control of fungal rot on apples".

Abstract:

The efficacy of plant essential oils was evaluated on apples cv Golden Delicious, Granny Smith, Red Chief and Royal Gala in postharvest to control *Botrytis cinerea* and *Penicillium expansum*. The essential oils from basil (*Ocimum basilicum*), fennel (*Foeniculum sativum*), lavender (*Lavandula officinalis*), marjoram (*Origanum majorana*), oregano (*Origanum vulgare*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), savory (*Satureja montana*), thyme (*Thymus vulgaris*) and wild mint (*Mentha arvensis*) were tested at different concentrations. All the essential oils are from mediterranean plants. The fruits were artificially wounded and inoculated with a suspension at 1 x 10^5 conidia mL^{-1} of each pathogen. After 12 hours, emulsions at 1% and 10% of each essential oil were dropped into each inoculated wound. A tebuconazole chemical control and an inoculated control were also included. All the treated fruit were stored at 4±1°C. After 15 and 30 days, the diameter of the rot around each wound was measured. Results revealed that efficacies of the essential oils tested are cultivar and storage time dependent. In any case, the treatments with essential oils from oregano, savory and thyme showed a significant efficacy in all apple cultivars tested. The treatments with essential oil emulsions at 10% were phytotoxic for all evaluated cultivars of apples.

Key words: Antifungal activity; *Botrytis cinerea*; Essential oil; *Penicillium expansum*; Postharvest rot.

1. Introduction

Blue and grey mould are caused on apple by *Penicillium expansum* and *Botrytis cinerea* respectively[1]. Both microorganisms are well-known pathogens on apple, and during postharvest they are particularly severe even in production areas where the most advanced storage technologies are available[2]. In North-Western Italy, the presence of *B. cinerea* and *P. expansum* has been reported among the most common fungi causing apple rot in storage[3]. Amiri and Bompeix[4] evaluated the presence of *Penicillium* spp. in apple storage rooms in France and reported that up to 62% of the isolates were *P. expansum.*

The use of several synthetic fungicides available at present is limited by emergence of resistant strains[5-6]. Following intensive use, resistance to thiabendazole has been reported in *Penicillium* and *Botrytis* populations[7]. Also the public concern over the health and environmental hazards associated with high levels of pesticide use in fruit orchards[8-9] and the lack of continued approval of some of the most effective fungicides[10], have generated interest in the development of alternative nonchemical methods of postharvest control.

The general antifungal activity of essential oils is well documented [11-12-13]. Most of the essential oils have been reported to inhibit postharvest fungi *in vitro*[14-15-16] and the antifungal activity of different compounds isolated from essential oils has been also reported[17-18-19]. The use of these volatile compounds has acquired increasing interest in the recent years. On apples, the activity of the monoterpene eugenol (the most significant active component of clove oil) was evaluated against *B. cinerea*, *M. fructigena, P. expansum* and *P. vagabunda*[20]. Carvacrol (one of the major constituents in oregano essential oil) is a phenol that has shown a high inhibition of mycelial growth of *Neofabraea alba* on apples[21].

Some of the essential oils have been reported to protect stored commodities from deterioration[22]. The bioactivity in the vapour phase of essential oils was recognized as a characteristic that makes them attractive as possible fumigants for stored product protection. The fungitoxic activity of the essential oils may be due to synergism among their components, since the most of the fungicides activity has been reported to be enhanced when combined[23-24].

The aim of this study was to assess the efficacy of different plant essential oils, at different concentrations, in postharvest control of *B. cinerea* and *P. expansum*, on different cultivars of apples.

2. Materials and methods

2.1. Pathogen preparation

Two strains of *B. cinerea* and two of *P. expansum* were isolated from rotten apples and tested for their virulence by inoculating in artificially wounded apples. They were used as a mixture throughout the work, to ensure a high level of disease and the presence of rots caused by both pathogens. Each strain was stored in slants on potato dextrose agar (Merck, Darmstadt, Germany) with 50 mg L^{-1} of streptomycin (Merck, Darmstadt, Germany) at 4°C. Spore suspensions were prepared by growing the pathogens on PDA Petri dishes added with 50 mg L^{-1} of streptomycin. After two weeks of incubation at 25°C, spores from the two strains of each pathogen species were collected and suspended in sterile Ringer's solution (Merck, Darmstadt, Germany). After filtering through eight layers of sterile cheese-cloth, spores were counted and brought to a concentration of 1 x 10^5 spores mL^{-1} for each pathogen. The resultant suspensions were shaken with a vortex mixer for 30 seconds before inoculation.

2.2. Essential oil preparation

The essential oils from basil (*Ocimum basilicum*), fennel (*Foeniculum sativum*), lavender (*Lavandula officinalis*), marjoram (*Origanum majorana*), oregano (*Origanum vulgare*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), savory (*Satureja montana*), thyme (*Thymus vulgaris*) and wild mint (*Mentha arvensis*) as commercial preparations with a 99% purity, were purchased from SOAVE (Turin, Italy). A 10% emulsion (10% essential oil, 88% sterilized water and 2% Tween20 (Merck, Darmstadt, Germany) as emulsifying agent) and a 1% emulsion (10% essential oil emulsion at 10% and 90% sterilized water) were prepared from each essential oil. All the resultant emulsions were shaken for 30 seconds before application for obtain an homogeneous essential oil mixture.

2.3. Efficacy of essential oil treatments

Apples (*Malus domestica*, cv Golden Delicious, Granny Smith, Red Chief and Royal Gala), harvested in northern Italy orchards following integrated pest management practices, were divided in groups of 15 fruits per treatment. All the fruits, free from evident wounds and rot, were disinfected in a sodium hypochloride solution at 1% and rinsed under tap water, dried at room temperature and punctured with a sterile plastic tip at the equatorial region (3 mm depth; 3 mm wide; 3 wounds per fruit). Ten microliters of pathogen suspension (1 x 10^5 spores mL^{-1}) were dropped into each wound. Apples were kept at room temperature for 12 hours for helping the establishment of the pathogen. Then, ten microliters of essential oil emulsion were dropped into each inoculated wound. A tebuconazole suspension, made with 0.5 g of water dispersible granule of Folicur WG® (Bayer CropScience, Milan, Italy) with 25% tebuconazole in one liter of water, was used as a chemical control by dropping ten microliters of suspension into each inoculated wound. An inoculated control was also performed. All treatments, the chemical control and the inoculated control were stored in cold chambers at 4±1°C for 30 days. The diameter of the rot around each wound was measured after 15 and 30 days of storage. Each trial was performed at least twice.

2.4. Statistical analysis

Data of the two repetitions were pooled together and the statistical analysis was performed by ANOVA (one – way analysis of variance) with SPSS-WIN software and the Duncan's multiple range test was employed at $P < 0.05$.

3. Results

3.1. Efficacy against grey mould

The efficacy of treatments with essential oils against *B. cinerea* is summarized in Figure 1. The decay caused by this pathogen at 4±1°C was significantly higher on apples cv Golden Delicious compared to the other apples cultivars. This situation caused a premature end of both repetitions of the trial after 15 days of storage. However, it was possible to observe that the efficacy of the treatment with thyme essential oil at 10% was the only one statistically similar to the chemical control (Fig. 1A). Among the treatments with 1% essential oil, the thyme one also showed the highest efficacy.

After 15 days of storage on apples cv Granny Smith, the efficacy of the treatments with oregano, savory, and thyme essential oils at 1% resulted statistically similar to chemical control. The same was observed with the treatments with fennel, lavender, marjoram, wild mint, rosemary, and sage essential oils at 10% (Fig. 1B). The treatments with basil, peppermint, oregano, savory, and thyme essential oils at 10% showed an efficacy not statistically different to the chemical control. After 30 days of storage, the treatments with basil, fennel, lavender, peppermint, oregano, and sage essential oils at 10% showed an efficacy statistically similar to that offered by the chemical control. Only the treatments with savory and thyme essential oils at 10% showed an efficacy not statistically different to the chemical control also after 30 days of storage.

On apples cv Red Chief, the efficacy of the treatment with savory essential oil at 1% resulted not statistically different to chemical control after 15 days of storage (Fig. 1C). Between the treatments at 10%, only the efficacy of the treatments with wild mint and rosemary essential oils was statistically different to chemical control. After 30 days of storage, the efficacy of the treatments with basil, lavender, oregano, sage, savory, and thyme essential oils at 10% was statistically similar to chemical control. The treatment with marjoram essential oil at 10% showed a higher efficacy than that provided by the chemical control, but remains statistically similar. Instead, all the treatments with essential oils at 1% showed an efficacy statistically different from the chemical control.

After 15 days of storage on apples cv Royal Gala, the efficacy of the treatment with thyme essential oil at 1% resulted statistically similar to the chemical control. The same results were observed with the treatments with basil, fennel, lavender, wild mint, oregano, and savory essential oils at 10% (Fig. 1D). The treatment with thyme essential oil at 10% showed an efficacy statistically higher than that showed by the chemical control. After 30 days of storage, the treatment with thyme essential oil at 1% and 10% and basil and oregano essential oil at 10% showed an efficacy statistically similar to the chemical control.

3.2. Efficacy against blue mould

The efficacy of treatments with essential oils against *P. expansum* is summarized in Figure 2. On apples cv Golden Delicious, after 15 days of storage, all the performed treatments showed an efficacy statistically different from the chemical control (Fig. 2A). The same results were observed after 30 days of storage.

After 15 days of storage, on apples cv Granny Smith, all the treatments but those with fennel, wild mint, rosemary, and sage essential oils at 1% and those with lavender, sage, and savory essential oils at 10% showed an efficacy not statistically different to chemical control (Fig. 2B). After 30 days of storage, all treatments showed an efficacy statistically different from the chemical control.

On apples cv Red Chief, the treatment with savory essential oil at 1% and the treatments with basil, fennel, lavender, marjoram, peppermint, oregano, sage, savory and thyme essential oils at 10% showed an efficacy statistically similar to that provided by the chemical control after 15 days of storage (Fig. 2C). Instead, after 30 days of storage, the efficacy of the treatment with marjoram essential oil at 10% was higher than that showed by the chemical control, although not statistically different. All the treatments with essential oils at 1% showed an efficacy statistically different from the chemical control.

The treatment with the essential oil of thyme at 10% reported an efficacy statistically similar to the chemical control on cv Royal Gala after 15 days of storage (Fig. 2D). Instead, after 30 days of storage, the efficacy of the treatments with essential oils of thyme at 1% and 10%, basil and oregano at 10% was statistically similar to chemical control.

4. Discussion

The essential oils tested proved their antifungal activity as postharvest treatments against *B. cinerea* and *P. expansum* on apples. The efficacy of the treatments with essential oils depended on the natural resistance of apple cultivars to *B. cinerea* and *P. expansum*. Against both pathogens, the treatments with savory and thyme essential oils at 1% showed a higher efficacy on apples cv Granny Smith and cv Red Chief (strongly less susceptible to attacks in postharvest) than on apples cv Golden Delicious and cv Royal Gala. Treatments with essential oils from basil on apples cv Granny Smith and from fennel on apples cv Golden Delicious resulted statistically different from the chemical control, but showed an efficacy widely higher than the other essential oil treatments tested.

The length of storage time could also influence the antifungal activity of the essential oil treatments. On apples cv Granny Smith, the efficacy of the treatment with marjoram essential oil at 1% against *P. expansum* was higher after 15 days than after 30 days of storage at 4.0±1°C. A similar situation was observed with the treatment with savory essential oil at 1% against *P. expansum* on apples cv Red Chief and with the treatment with oregano essential oil at 1% against *B. cinerea* on apples cv Granny Smith. This situation could suggest that treatments with essential oils should be used for short storage times or should be repeated after a defined time period depending on the fruit cultivar.

The variability of composition for some essential oil-producer species considered (Table 1.) certainly determines the antifungal activities of the essential oils. The achieved results permit to narrow the future application of essential oils (or their active compounds) on apples in postharvest. Carvacrol and thymol are the major components in oregano essential oil, as *p*-cymene and carvacrol in savory essential oil, and thymol and *p*-cymene in thyme essential oil[31-54]. The different efficacy between the treatments is due to the fact that the antifungal activity of essential oils depends on the fungitoxic properties of the most significant active components and their synergy. This situation also means that the possible phytotoxic effects of treatments with essential oils may be due to the same active components in each essential oil. In this study, the treatments performed with all essential oils tested at 10% were phytotoxic for the carposphere of all apple cultivars tested. The size of the lesions was proportional to the efficacy showed by the treatments with essential oils at 10%. This means that a higher efficacy on pathogen control could produce a higher damage on the carposphere of apples. The fruits most susceptible to this side effect were apples cv Golden Delicious, followed by apples cv Granny Smith.

The antifungal activity of essential oils could be enhanced by the application method. The potential of using essential oils by dipping or spraying to control postharvest decay has been examined in fruit and vegetables[58-59-60]. Also the combination with other postharvest treatment could improve the efficacy of control of postharvest pathogens[61-62]. Under controlled conditions, this study assess the efficacy of essential oils with the purpose to select the best ones and their best concentration for apples. Future analysis should involve the application of essential oils by dipping or spraying under semi-commercial conditions, including storage in controlled atmosphere of a larger number of fruits.

Nonetheless, the selection of an essential oil for a postharvest treatment must be based mainly on the characteristics of the fruit, desirable storage time and decay to control.

Some specific compounds of essential oils (i.e. Eugenol, 1,8 Cineole, Menthol, Cinnamaldehyde, Pulegone, α- and β-Thujone, α- and β-Pinene, Linalyl acetate) have been reported as toxic for humans[63-64-65], generally present in many essential oil chemotypes. Future research in postharvest should include the analysis of the withholding period of essential oil treatments, as used on synthetic fungicides, and the possible traces of toxic compounds on treated fruit. Since biochemical and environmental factors influence aroma and flavour of apples[66], the application of postharvest treatments with essential oils should take in consideration the assessment of the normal balance between apple volatile compounds such as alcohols, aldehydes, carboxylic esters, ketones, and ethers.

Acknowledgements

This research was funded by the projects "CIPE -- Production of stone fruit in Piedmont: monitoring, prevention and control of pathogenic and mycotoxigenic fungi to guarantee food safety" and "DRUMP -- Drupacee minori in Piemonte: problemi fitopatologici e difesa post-raccolta" granted by the Piedmont Region.

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Table 1.

Major components of essential oils reported in literature.

Essential oil	Main compound	Other compounds *	References
Basil	Linalool	Cinnamic acid methyl ester	Zhang et al., 2009 [25]
		Eugenol	De Almeida et al., 2007 [26]
		Methylchavicol	Shatar et al., 2007 [27]
Fennel	*trans*-Anethole	Fenchone	Telci et al., 2009 [28]
			Anwar et al., 2009 [29]
			Cosge et al., 2008 [30]
Lavender	Linalool	Linalyl acetate	Cong et al., 2008 [31]
		Geraniol	Fakhari et al., 2005 [32]
		Lavandulyl acetate	Evandri et al., 2005 [33]
Marjoram	Terpinen-4-ol	*cis*-Sabinene hydrate	Busatta et al., 2008 [34]
		γ -Terpinene	Vagi et al., 2005 [35]
		Linalool	Vera et al., 2009 [36]
Oregano	Carvacrol	Thymol	De Barros et al., 2009 [37]
		p-Cymene	Azizi et al., 2009 [38]
			Prieto et al., 2007 [39]
Peppermint	Menthol	*p*-Menthone	Dwivedi et al., 2004 [40]
		Menthyl acetate	Shahi et al., 1999 [41]
		1,8-Cineole	Rohloff et al., 1999 [42]
Rosmary	Camphor	1,8-Cineole	Djeddi et al., 2007 [43]
		2-Ethyl-4,5-Dimethylphenol	Katerinopoulos et al., 2005 [44]
		Borneol	Touafek et al., 2004 [45]

Sage	1,8-Cineole	Camphor α -Thujone	Raal et al., 2007 [46] Maksimovic et al., 2007 [47] Vukovic-Gacic et al., 2006 [48]
Savory	*p*-Cymene	Carvacrol Thymol	Prieto et al., 2007 [39] Fraternale et al., 2007 [49] Mastelic et al., 2003 [50]
Thyme	Thymol	Carvacrol *p*-Cymene γ -Terpinene	Vurro et al., 2009 [51] Dawidowicz et al., 2009 [52] Chizzola et al., 2008 [53] Bagamboula et al., 2004 [54]
Wild mint	Menthol	Pulegone Isomenthone	Mkaddem et al., 2009 [55] Phatak et al., 2002 [56] Singh et al., 2005 [57]

* Only compounds reported over 10% of the essential oil composition were considered.