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Investigating the Effects of Plant Essential Oils on Post-Harvest Fruit Decay

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Additional information is available at the end of the chapter

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Abstract

Essential oils are one of the most important natural products derived from plants, due to their various biological properties and their medicinal and nutritional uses. This chapter provides an overview of several different aspects relating to essential oils including a historical perspective, the uses of essential oils, their main sources and antifungal activity, their bioactive single constituents and their modes of action. The chapter will also give an insight into the chemical measures necessary for controlling plant pathogens and their negative impact on human health and/or the environment. It will also review the different sources of essential oils such as sage, oregano, thyme and marjoram from the *Lamiaceae* family, vervain from the *Verbanaceae* family, and magnolia from the *Magnoliaceae* family. The antimicrobial activity of essential oils is reviewed, with particular emphasis on the antifungal properties exhibited against some serious pathogenic fungi and post-harvest disease. Moreover, various antimicrobial tests and techniques, such as various kill-time studies, killing time determination, LD₅₀ and growth curve recording, poisoned food techniques, spore germination and measurement of metabolic CO₂ are included. Finally, five case studies relating to the antifungal activity of some plant essential oils, either *in vitro* or *in vivo*, against post-harvest pathogenic fungi are reviewed at the end of this chapter.

Keywords: Plant essential oils, Antifungal activity, Post-harvest diseases, Chemical measurements, Antimicrobial tests, Fungal pathogenicity

1. Introduction

An essential oil is a concentrated hydrophobic liquid containing volatile aromatic compounds from plants. Essential oils are also known as volatile oils or ethereal oils. An oil is "essential" in the sense that it carries a distinctive essence of the plant. It does not form a distinctive category for either medical, pharmacological, or culinary purposes.

Essential oils have several applications in the manufacture of perfumes, cosmetics, and soaps. In the food industry, essential oils can be used for flavouring, and for adding scents to incense. The use of some essential oils as alternative antimicrobial agents, replacing chemical treatments, has attracted considerable interest from post-harvest scientists in recent years. Most essential oils and their single constituents have been reported to inhibit post-harvest fungi both *in vitro* and *in vivo*. The use of these volatile compounds has garnered a great deal of interest recently.

There is no doubt that essential oils have important pharmacological properties, which result in their wide use in pharmaceutical practices. The first investigation into the antimicrobial activity of essential oils was a study by Buchholtz [1] who found that thymol has higher growth inhibitory properties on bacteria having been cultivated in a tabac decoction than phenol, which was previously considered to be the best systematic substance for use as surgical antiseptic [2]. Therefore, a great deal of research has been carried out aimed at studying the antimicrobial activities of several essential oils that have already been registered in the pharmacological industry.

The fungitoxic activity of essential oils may be due to synergism among their components, since most of this activity has been reported to be enhanced when combined [3]. Bioactivity in the vapor phase of essential oils was recognized as a characteristic that makes them attractive for use as possible fumigants for the protection of stored product [3–6].

This chapter will address different aspects relating to plant essential oils and fungal pathogenicity ranging from the chemical use in controlling plant pathogenic fungi as well as discussing the main sources of essential oils, and their biological activity. Furthermore, this chapter will provide an insight into the mode of action of single constituents of different essential oils, using different case studies.

2. Chemical control of plant pathogenic fungi

Collectively, fungi and fungal-like organisms cause more plant diseases than any other group of plant pathogens, with over 8,000 species shown to cause disease. The importance of fungi as agents of plant and human diseases, producers of industrial and pharmacological products, and as decomposers, has prompted scientists worldwide to study their biology. Some of the world's great famines and human suffering can be blamed on plant pathogenic fungi [7].

Chemical disease control employs the use of chemicals that are generally toxic to pathogens and characterized by their specific effect, such as many commercial fungicides and antibiotics. Basically, any chemical agent must be effective and act safely, without harming any live organism and must also have minimal or no effect on the environment, microflora and soil.

Chemical fungicides are generally used in the control of fungal diseases.

Recently, growing public concern over the health and environmental hazards associated with the increased levels of chemical pesticides and the lack of approval for the renewal of some of

the most effective active molecules, has led to the development of safe, alternative, and natural methods of post-harvest disease control [8]. To minimize the development of pest resistance as a result of the overuse of chemical fungicides, it was advisable to use a variety of fungicides belonging to different chemical groups. Throughout the 1980s and 1990s, public concern about the use of agricultural chemical pesticides grew as a result of the high risk of poisoning to all living organisms, beneficial micro-flora and micro-fauna, and the contamination of food products. The risk from chemical pesticides is due to the possibility that they may be absorbed through the skin, inhaled or ingested through consumption of contaminated plants.

To overcome the risks of chemical pesticides, some precautions should be taken with respect to the handling of the chemicals and the management of agriculture practices. Environmental concerns focus mainly on protectant fungicides such as copper and sulfur sprays, which have the potential to affect a broad range of organisms if they are washed off leaves and then accumulate in the soil or are washed into waterways. A lot of countries have already started educational programs for farmers to reduce the overuse of chemical pesticides, and hence increase the productivity of several economic crops.

Recently, there has been a great interest in the use of essential oils and plant extracts as possible natural substitutes for conventional synthetic pesticides. This may be attributed mainly to ecosystem pollution and pesticide resistance in pests, insects and fungal pathogens.

3. Plant essential oils

Essential oils are volatile compounds produced in many species of plant. These oils are thought to play a role in plant defense mechanisms acting against phytopathogenic microorganisms [9–13]. Subsequently, plant essential oils were subjected to pharmacological studies and, later, to numerous and frequent tests of their antimicrobial activity.

Several methods have been used to evaluate the *in vitro* antimicrobial activity of different essential oils. Among the most common methods are agar diffusion tests, serial broth or agar dilution tests, and vapor phase tests [14].

Further antimicrobial tests comprise the following:

1. Various kill-time studies:

This test deals with determining the activity of a compound relative to the activity of phenol, after 15 min (phenol coefficient).

2. Killing time determination:

This test deals with determining the exact time needed for the complete inhibition of the target organism after contact with the test compound, using contaminated silk threads.

3. LD-50 and recording of growth curves:

This test records growth curves and determines the amount of a compound effectively inhibiting the growth of 50% of test organisms.

4. Poisoned food techniques:

Tests in which the delay of microbial growth is determined in the presence of growth inhibitors.

5. Spore germination:

This test is suitable for use with fungi, especially during short contact time studies.

6. Measuring of metabolic CO₂:

This test monitors the presence or absence of the growth of yeast, or visualizes growth using indicators such as sulfur salts from sulfur supplemented cow milk as a growth medium, 2,3,5-triphenyltetrazolium chloride, or p-iodo-nitrophenyltetrazolium violet.

3.1. Main sources of plant essential oils

1. Family Lamiaceae

Sage

Sage is considered to be the main genus among the *Lamiaceae* family, which consists of about 900 species widely distributed in the temperate, subtropical and tropical regions all over the world but especially in the Mediterranean region, central Asia, central and South America, and in southern Africa. Globally, the best known species of the family, used in both traditional and modern medicine are *Salvia officinalis*, *S. fruticosa* and *S. divinorum*.

Another important plant is oregano, considered to be the most valued species worldwide. About 60 plant species were listed within this common name. The majority of oregano species belong to the *Lamiaceae* and *Verbenaceae*.

Oregano

Oregano is a perennial herb; its flowers are purple, 3–4 mm long, and produced in erect spikes. It is sometimes called wild marjoram, and its close relative *Origanum majorana* is known as sweet marjoram. Many subspecies and strains of oregano have been developed by humans over the centuries for their unique flavors or other characteristics. It is an important culinary herb, used for the flavor of its leaves, which can actually be more flavorful when dried than fresh. It has an aromatic, warm and slightly bitter taste, which can vary in intensity.

The antifungal and antibacterial activity of oregano essential oil against a number of plant pathogens has been reported by Adebayo et al. [15] These pathogens include *Aspergillus niger* v. Tieghem, *A. flavus* Link, *A. ochraceus* Wilhelm, *Fusarium oxysporum* Snyder and Hansen, *Penicillium* sp. L., *Pseudomonas aeruginosa* Schroter ATCC 2730, *Staphylococcus aureus* Rosenbach ATCC 6538, *F. solani* var. *coeruleum* (Martius) Saccardo, *Clavibacter michiganensis* S., *Phytophthora infestans* Mont., *Sclerotinia sclerotiorum* Lib. and *Xanthomonas vesicatoria* D.

Thymes

Thyme is an evergreen herb with culinary, medicinal and ornamental uses. The most common variety is *Thymus vulgaris*. Thyme belongs to the genus *Thymus* of the mint family (*Lamiaceae*) and is a relative of the oregano genus *Origanum*.

The essential oil of *T. capitatus* Hoffm. & Link, displayed antifungal activity in stored foods and inhibited the growth of both *B. cinerea* and *Monilinia fructicola* [16]. *T. vulgaris* showed antifungal activity against some post-harvest fungal pathogens such as *B. cinerea*, *P. italicum*, *P. citrophthora* and *Rhizopus stolonifer* [10].

Marjoram

Marjorana hortensis (Lamiaceae), commonly known as marjoram, is a perennial herb or under-shrub with sweet pine and citrus flavors. It has a long history of medicinal and culinary use.

Camele et al. [10] reported that the last two essential oils of Lamiaceae family showed antifungal activity against *P. citrophthora* and *R. stolonifer*. *M. hortensis* showed antifungal activity against *C. acutatum* and *B. cinerea*, and antibacterial activity against two strains of G+ve (*Bacillus megaterium* and *C. michiganensis*) and five strains of G-ve (*Escherichia coli*, *X. campestris*, *B. mojavensis*, *P. savastanoi* and *P. syringae* pv. *phaseolicola*) (Elshafie et al., data not published).

2. Family Verbanaceae

Vervain

Verbena officinalis (Verbenaceae) commonly known as vervain, is a perennial medicinal plant which grows natively in Europe. It is widely naturalized outside its native range, for example in North America. It has been used traditionally as folk medicine in some countries, and recently was reported to act as an anticancer agent. Despite its widespread uses, the mechanisms of the pharmacological actions of the herb are still unclear.

The antimicrobial activity of vervain essential oil was reported recently by Elshafie et al. [12] who found that this essential oil significantly reduced (*in vivo*) the brown rot lesion diseases of peach caused by *M. laxa*, *M. fructicola* and *M. fructigena*.

3. Magnoliaceae

Magnolia

Magnolia is a large genus of about 210 flowering plant species in the family Magnoliaceae. Its trees are very ancient and the flowers and oil have been used in many cultures around the world.

M. liliflora essential oil showed potential *in vitro* and *in vivo* antifungal effects against *B. cinerea*, *Colletotrichum capsici* (Syd.) E.J. Butler & Bisby, *F. oxysporum* Snyder and Hansen, *F. solani*, *P. capsici* Leonian, *Rhizoctonia solani* (Cooke) Wint. and *S. sclerotiorum* [17].

The essential oil of *M. liliflora* also showed a potential *in vivo* antifungal effect against *P. capsici*, and this activity could be attributed to its constituents: α -terpineol, α -bourbonene, β -caryophyllene, 2- β -pinene, α -humulene, farnesene, and caryophyllene oxide components [17].

3.2. Antimicrobial activity of plant essential oils

Numerous essential oils have shown an antifungal effect against several post-harvest pathogens, e.g. *B. cinerea* Pers. [18–20], *Aspergillus* spp. [21–23], *Fusarium* spp. [24,25], *Penicillium* spp.

[26], *R. stolonifer* (Ehrenb.: Fr.) Vuill. [27,28], *C. gloeosporioides* Penz. [29]. The antifungal effect is attributed mainly to the inhibition of both mycelial growth and spore germination. This hypothesis suggests that the expression of disease will be restricted by the impeding of the initial infection and the subsequent mycelial spread beyond the infection site [30].

Clove oil is an essential oil extracted from the clove plant, *Syzygium aromaticum* (L.) Merr. & Perry, and has been reported to act as a bioactive substance — especially its active component monoterpene eugenol [31] — against *B. cinerea*, *M. fructigena* Honey, *P. expansum* Link and *Phlyctema vagabunda* Desm. in apples. Carvacrol (one of the major constituents of oregano essential oil) is a phenol that was reported to show a high inhibition of mycelium growth in *Neofabraea alba* (E.J. Gutrie) Verkley on apples [32].

Essential oils from basil (*Ocimum basilicum* L.), fennel (*Foeniculum sativum* Mill.), lavender (*Lavandula officinalis* Chaix), marjoram (*O. majorana* L.), oregano (*O. vulgare* L.), peppermint (*Mentha piperita* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), savory (*Satureja montana* L.), thyme (*T. vulgaris* L.) and wild mint (*Mentha arvensis* L.) showed a potentially significant antifungal activity higher than that available from chemical treatments in post-harvest treatments against *B. cinerea* and *P. expansum* on apples [3].

The different efficacies of essential oils are due to the differing fungitoxic properties of each single active constituent, as well as the synergic effect [3,32]. This hypothesis suggests that the possible phytotoxic effects of essential oil treatments may be due to the same active components existing in each essential oil. The length of storage may also negatively influence the antifungal activity of the essential oil treatments. Therefore, treatments using essential oils should only be used for short storage times, or they should be repeated after a defined time period has elapsed, depending on the fruit cultivar in question [3]. The selection of an essential oil for a post-harvest treatment must be based mainly on the characteristics of the fruit, desirable storage time and decay.

The antifungal activity of essential oils could be enhanced by the method of application. The potential of using essential oils by dipping or spraying to control post-harvest decay has already been examined in fruit and vegetables [33–35]. The combination of various post-harvest treatments may improve the efficacy of controlling post-harvest pathogens [30,36,37].

Essential oils from thyme (*T. capitatus* L.), spearmint (*M. spicata* L.) and anise (*Pimpinella anisum* L.) exhibited inhibitory effects on the development of *M. fructicola* (G.Winter) Honey [36,37]. The essential oil of Lemon myrtle (*Backhousia citriodora* F.Muell.) has been reported to have antifungal activity against *M. fructicola*, and has been shown to have a strong antimicrobial activity, mainly with respect to its potential application as a topical pharmaceutical product. Its main constituent, citral, had also been reported to exhibit a fungitoxic effect against a range of post-harvest pathogens [38].

Mechanism of antifungal activity of essential oils

Apart from the positive effect of each of the chemical constituents of different essential oils, several different studies have indicated that there seems to be a synergetic effect between the individual chemical constituents. This synergism in the aromatic plants components functions to make them more effective and reduces the developing resistance of any pathogenic fungi.

In particular, some single constituents such as carvacrol, γ -terpinene and p-cymene become more effective when they are combined together and act synergistically [15].

On the other hand, p-cymene is efficient facilitator of the transport of carvacrol across cell wall components and the cytoplasmic membrane of pathogenic fungi. Thymol, eugenol and carvone are widely used in the control of several fungi, particularly those which contaminate various important economic crops [13].

Another hypothesis suggested by Soylu et al. [39,40], is that the observed diameter reduction and lyses of the hypha wall, may be attributed to the enzymatic reactions within the essential oil which act to regulate synthesis of the wall. Furthermore, the lipophilic properties of the above mentioned single components might have the ability to degrade the plasma membrane, and thus to increase the permeability of the cytoplasm.

The following will give an insight into some case studies relating to the antimicrobial activity of some Mediterranean plant essential oils:

1st case study: *Biochemical characterization of oregano essential oil;*

2nd case study: *Antimicrobial activity of oregano, thyme and vervain essential oils;*

3rd case study: *In vivo antifungal activity of thyme and vervain essential oils;*

4th case study: *In vitro antifungal activity of the main components of vervain, thyme and oregano essential oils;*

5th case study: *In vitro and in vivo antifungal activity of single constituents of oregano essential oil.*

3.3. Case studies

First case study

Biochemical characterization of oregano essential oil

Mancini et al. [11] studied the biochemical characterization of *O. vulgare* ssp. *hirtum* essential oil from the Southern Apennines (Italy). *O. vulgare* is composed mainly of phenolic compounds belonging to the carvacrol/thymol chemotype. The possible inhibitory fungicidal activity of *O. vulgare* was determined against *M. laxa* (Aderh. & Ruhland) Honey, *M. fructigena* and *M. fructicola* as follows: prepare different concentrations of each oil + potato dextrose agar (PDA) + (0.2 %) with 0.2 % Tween 20 and 250, 500, and 1000 ppm of each essential oil under study, then pour 14 ml of PDA+ oil in Petri dish.

Following that, completely dry off the preparation under laminar flow, 0.5 cm disc from the studied fungi 96 h old was inoculated in the centre of each Petri dish. All plates were incubated at 22°C for 96 h in the absence of light, and the diameter of any fungal mycelium growth was measured in mm. PDA plates + Tween 20 without oils were inoculated with the same fungi as a control. Fungitoxicity was expressed as the percentage of growth inhibition (PGI) and calculated according to Zygodlo et al. [41] as follows:

$$PGI\ (\%) = 100 \times (GC - GT) / GC$$

Where GC represents the average diameter of fungi grown in PDA (control); GT represents the average diameter of any fungi cultivated on the treated PDA containing the essential oil.

The oils tested have shown antifungal activity against *M. laxa*, *M. fructigena* and *M. fructicola* (Table 1) [11] and have neither shown any phytotoxic activity against germination and initial radicle elongation of *Sinapis arvensis* L., *Phalaris canariensis* L., *Lepidium sativum* L. and *Raphanus sativus* L., nor have they shown any haemolysing effect against the cell membrane of bovine erythrocytes [11].

Inhibition Percentage (PGI %)				
Treatments		<i>M. laxa</i>	<i>M. fructigena</i>	<i>M. fructicola</i>
<i>O. vulgare</i> (M)	1000 ppm	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
	500 ppm	79.6 ± 2.21b	69.2 ± 1.67c	54.3 ± 2.65c
	250 ppm	67.9 ± 1.10c	58.0 ± 0.83d	14.3 ± 6.19e
<i>O. vulgare</i> (MP)	1000 ppm	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
	500 ppm	74.2 ± 3.31c	62.1 ± 3.35cd	45.6 ± 2.65c
	250 ppm	48.4 ± 2.21d	38.4 ± 3.35e	20.6 ± 2.65de
<i>O. vulgare</i> (SGP)	1000 ppm	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
	500 ppm	92.9 ± 1.10a	86.9 ± 1.68b	85.00 ± 1.77b
	250 ppm	46.8 ± 4.42d	43.1 ± 3.34e	25.6 ± 0.88d

Where: M: Mandia sample; MP: Marconia di Pisticci sample; SGP: San Giovanni a Piro sample. Values followed by the same letter in each vertical column are not significantly different according to Tukey test at $P < 0.05$, data are expressed as mean (SDS), R=3.

Table 1. Antifungal activity of *O. vulgare* essential oils from three different localities.

Second case study

Antimicrobial activity of oregano, thyme and vervain essential oils

Adebayo et al. [15] reported that oregano essential oil has antimicrobial activity against a number of plant pathogens such as *A. niger* van Tieghem, *A. flavus* Link, *A. ochraceus* Wilhelm, *F. oxysporum*, *F. solani* var. *coeruleum* (Martius) Saccardo, *Penicillium* spp., *P. aeruginosa* Schroter ATCC 2730, *S. aureus* Rosenbach ATCC 6538, *C. michiganensis* (Smith) Davis, *P. infestans* Mont, *S. sclerotiorum* Lib. and *X. gardneri* Dowson. In addition, different species of oregano such as *O. vulgare* and *O. syriacum* L. showed antifungal activity against *B. cinerea* [33,34].

Thyme and vervain essential oils were obtained from *T. vulgaris*, *V. officinalis* L. have shown *in vitro* potential fungicidal activity against four causal agents of post-harvest orange fruit rot:

B. cinerea, *P. italicum*, *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian and *R. stolonifer* (Ehrenb.: Fr.) Vuill. [10].

Third case study

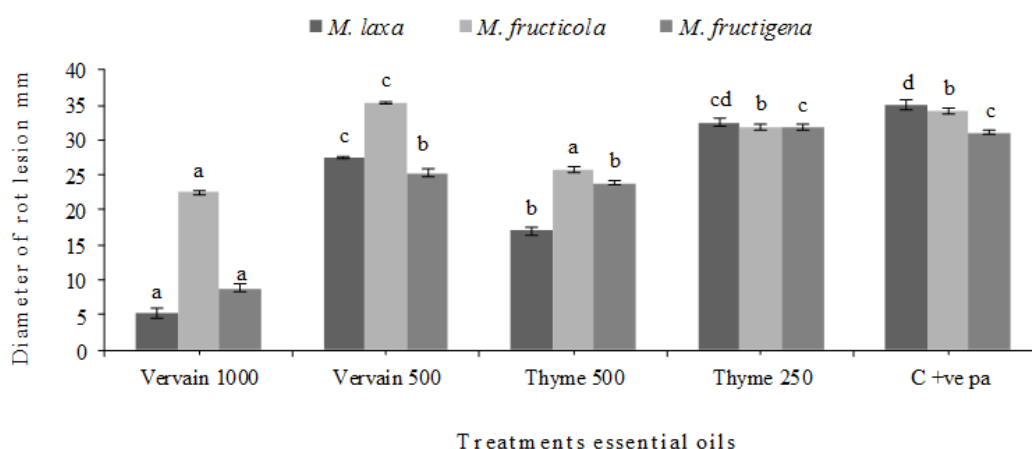
In vivo antifungal activity of thyme and vervain essential oils

Elshafie et al. [12] studied the *in vivo* antifungal activity of both thyme oil and vervain oil against post-harvest brown rot disease of peach. They reported that thyme oil was mainly composed of *o*-cymene (56.2%), while the main components of vervain oil were citral (44.5%) and isobornyl formate (45.4%) [10]. In addition, both oils were evaluated for their *in vivo* antifungal activity against the post-harvest pathogen *Monilinia* on peach fruits. All tested peach fruits of cv. "Springcrest", were not treated by either pre- or post-harvest chemical pesticides, and were sterilized superficially with a 2 % sodium hypochlorite solution and were later washed with sterile distilled water. They were finally air dried before being inoculated with the above mentioned three phytopathogenic fungi at room temperature by injuring the fruit surfaces and inoculation with about 10 μ L of fungal suspension of 10^6 spore/mL. Spore suspensions were prepared by adding two loopfuls of each fresh fungal mycelium (7–10 days old) to 10 mL of sterile distilled water before each of the suspensions was filtered and the concentration adjusted by serial dilution in sterile distilled water. One day after inoculation, each single fruit group was sprayed with different concentrations of thyme essential oil at 250 and 500 ppm, or at 500 and 1000 ppm of vervain essential oil. Each experiment was repeated twice. Four fruits, after being wounded with a sterile needle, were sprayed only with sterile distilled water and used as negative control, while four fruits for each oil concentration were not inoculated and were used instead as a control to determine the possible phytotoxicity. All the fruit series were kept in a moist chamber at a high relative humidity (about 95%) for 4 days at room temperature before being observed for eventual appearance of symptoms. The fungitoxicity effectiveness was expressed as the diameter of the brown rot lesion in mm on fruit with respect to control. Both oils studied showed a promising fungicidal effect *in vivo* on the post-harvest diseases of peach fruits such as brown rot lesion caused by *M. laxa*, *M. fructigena*, and *M. fructicola* (Figure 1,12).

Fourth case study

In vitro antifungal activity of the main components of vervain, thyme and oregano essential oils

Camele et al. [42] investigated the biological activity *in vitro* of the main components " β -fellandrene, β -pinene, camphene, carvacrol, citral, *o*-cymene, γ -terpinene and thymol" extracted from three Mediterranean aromatic plants (*V. officinalis*, *T. vulgaris* and *O. vulgare*), against five etiological agents of post-harvest fruit decay, *B. cinerea*, *P. italicum*, *P. expansum*, *P. citrophthora* and *R. stolonifer*. The possible fungistatic or fungicidal activity of each studied essential oil components was determined as follows: (A) putting single 3-mm-thick and 0.5-cm-diameter PDA plugs containing fungal mycelium onto the central part of the surface of Petri dishes containing PDA pre-treated with different concentrations (50, 150 or 250 ppm) of each tested single component dissolved in 0.2% Tween-20, (B) dropping, under axenic conditions, 10 μ L aliquots of single suspensions containing 1×10^4 conidia/mL of the single target microorganism species onto the central part of surface of Petri dishes containing PDA



Where: C+ve pa: is positive control where the fruits were challenged only with pathogens.

Figure 1. *In vivo* antifungal activity of thyme and vervain oil against postharvest brown rot disease of peach caused by *M. laxa*, *M. fruticola* and *M. fructigena*. Bars with different letters indicate means values significantly different at $P < 0.05$ according to Tukey test. Data are expressed as mean of three replicates \pm SD.

prepared with the same percentage of Tween-20 and the single three component concentration. Results showed that citral exhibited a fungicidal action against *P. citrophthora*; carvacrol and thymol showed a fungistatic activity against *P. citrophthora* and *R. stolonifer*. Thymol showed fungicidal activity against *P. italicum*. Citral and carvacrol at 250 ppm, and thymol at 150 ppm all stopped the growth of *B. cinerea*.

Fifth case study

In vitro and *in vivo* antifungal activity of the single constituents of oregano essential oil

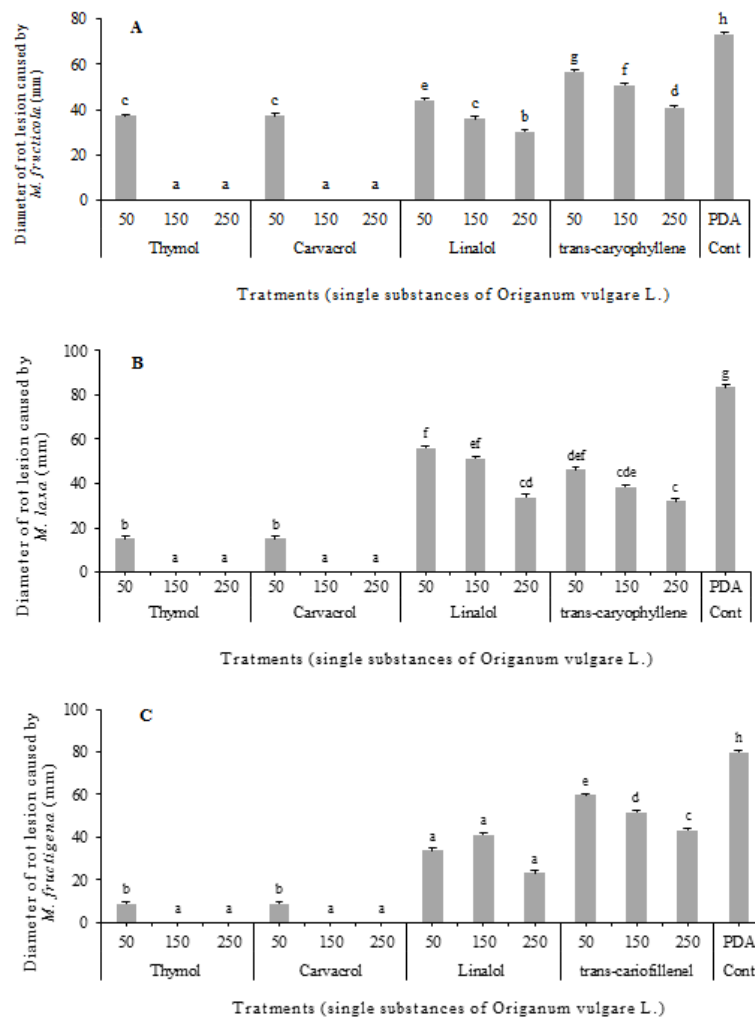
Elshafie et al. [13] evaluated the antifungal effect of the single components of *O. vulgare* ssp. *hirtum* essential oil against the post-harvest pathogens *M. laxa*, *M. fructigena* and *M. fruticola* *in vitro* and *in vivo*. The chemical characterization of *O. vulgare* reported by Mancini et al. [11] explained that it contains five main single components: carvacrol, thymol, linalool, citral and *trans*-caryophyllene [11].

In vitro antifungal activity

The possible fungicidal activity of the above five standards was determined according to the method of Soyulu et al. [40]. Three-mm-thick and 0.5-cm-diameter PDA plugs, axenically taken from the peripheral portion of basic colonies, were inoculated onto the central part of PDA Petri dishes pre-treated with different concentrations of each single component (50, 150 or 250 ppm) dissolved in 0.2% Tween 20. All plates were incubated at 22°C for 96 h in the absence of light. Negative controls comprised either PDA plates without any treatments, or PDA plates treated only with 0.2% Tween 20. The antifungal activity was expressed by measuring the diameter of any mycelium growth in mm [42].

Carvacrol and thymol have exhibited the highest activity during *in vitro* tests against all tested post-harvest *Monilinia* pathogens (Figure 2,13). Citral showed moderate antifungal activity,

lower than that of carvacrol and thymol. Linalool and *trans*-caryophyllene showed slight antifungal activity against all studied pathogens. On the other hand, thymol showed fungitoxic inhibition, whereas carvacrol and citral showed fungistatic activity.



Where: 50, 150 and 250 are the concentrations of each single substance in ppm; PDA is potato dextrose agar.

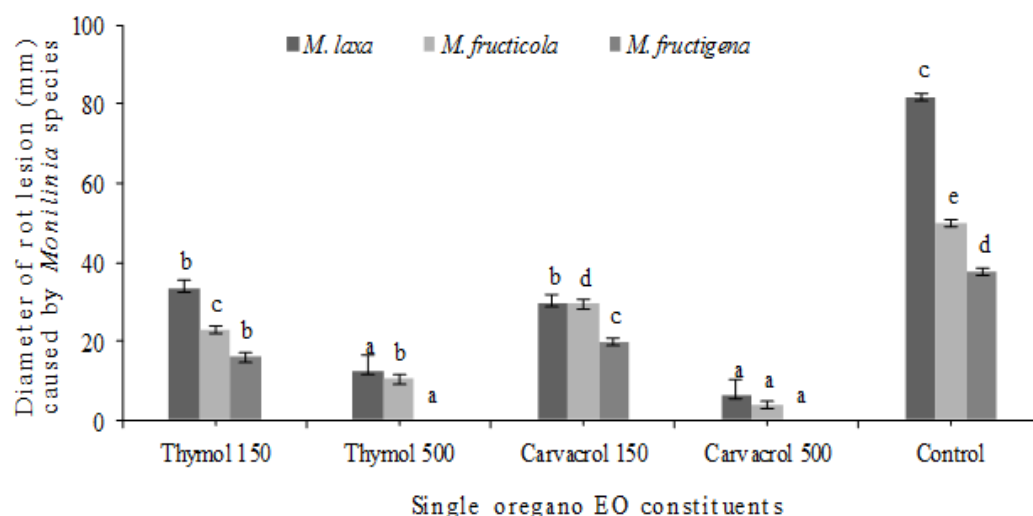
Figure 2. *In vitro* antifungal activity of the four single substances of *O. vulgare* oil against *M. laxa*, *M. fructicola* and *M. fructigena*. Bars with different letters indicate means values significantly different at $P < 0.05$ according to Tukey test. Data are expressed as mean of three replicates \pm SE.

In vivo antifungal activity

The bioactive treatments which exhibited *in vitro* activity were selected for evaluation of their *in vivo* activity against three *Monilinia* species causing brown rot of peach fruits, following the method of Hong et al. [43]. Tested peach fruits cv. "Springcrest", were not treated with either pre- or post-harvest chemical pesticides, and were superficially sterilized with 2 % sodium hypochlorite solution, then later washed with sterile distilled water, before they were finally, air dried and inoculated with the above mentioned three phytopathogenic fungi at room temperature. Each inoculum was performed by injuring the surface of the fruits with a sterile

needle and then adding 10 µl of fungal suspension containing 10^6 spore /ml. Liquid fungal cultures were prepared by adding two 3-mm-thick and 0.5-cm-diameter (4 days old) fungal discs to 150 ml of sterilized potato dextrose broth (PDB) medium. They were then incubated at 22 °C for 7–9 days. One day after inoculation, each fruit group was sprayed with an emulsion containing different concentrations of each single component at 150 or 500 ppm, dissolved in 0.2% Tween 20. The negative control composed of three groups of fruit sprayed only with sterile distilled water, whereas the positive control composed of three groups of fruits inoculated only with *Monilinia* isolates. The severity of symptoms induced by infection of the single *Monilinia* isolates was determined by measuring the diameter of brown rot lesions in mm after 3–5 days of incubation at room temperature (16–24 °C).

Carvacrol and thymol have shown a promising inhibition of the brown rot of peach fruits caused by *M. laxa*, *M. fructicola* and *M. fructigena* *in vivo* especially at a dose of 500 ppm (Figure 3,13). In particular, carvacrol showed the highest significant antifungal activity against *M. fructicola*.



Where: 150 and 500 are the concentrations of each single substance in ppm.

Figure 3. *In vivo* antifungal activity of thymol and carvacrol against brown rot disease of peach fruit caused by *M. laxa*, *M. fructicola* and *M. fructigena*. Bars with different letters indicate means values significantly different at $P < 0.05$ according to Duncan test. Data are expressed as mean of three replicates \pm SD.

4. Conclusions

Plant essential oils are one several promising environmentally friendly alternatives to conventional synthetic pesticides used to control several fungi and fungus-like organisms such as plant pathogens, food contaminants and decays. Most of the essential oils and their formulations mentioned in this review showed a high efficacy — either via direct contact or through

incorporation into nutrient media — against different fungal pathogens at very low concentrations when used either *in vitro* or *in vivo*. Among the essential oils used, oregano, thyme and vervain and their active constituents such as carvacrol, thymol, linalool, citral and their isomers, effectively inhibited mycelial growth and spore germination through fungistatic and/or fungicidal actions. The active ingredients of the essential oils and their isomers can be used effectively as seed and soil treatments — controlling most of the post-harvest decay fungi — and in plant and food protection.

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