

Current status of the effectiveness of essential oils for controlling phytopathogenic fungi, a review

Estado actual de la efectividad de aceites esenciales para el control de hongos fitopatógenos, una revisión

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ABSTRACT

Essential oils (EOs) are volatile plant derivatives that have been evaluated in the control of phytopathogenic fungi, and whose effectiveness has been associated with the part of the plant and concentration used. Hereby we provide an overview of the role of EOs on phytopathogenic fungi agents, presenting an overview of its diversity, constituents, tests under *in vivo* and *in vitro* conditions, their mechanisms of action, and the techniques to assess its effectiveness. The antimicrobial action of EOs is attributed to their ability to penetrate through cell membranes and inhibit the functional properties of fungal cells.

KEYWORDS

Hydrodistillation, secondary metabolites, *in vitro* and *in vivo* studies, direct contact and vapor phase techniques, coatings and bioactive films.

RESUMEN

Los aceites esenciales (AEs) son derivados volátiles de plantas que han sido evaluados en el control de hongos fitopatógenos y su efectividad se ha asociado con la parte de la planta y la concentración utilizada. Aquí presentamos una revisión del papel de los AEs en el control de hongos fitopatógenos, con una panorámica de su diversidad, constituyentes, pruebas bajo condiciones *in vivo* e *in vitro*, sus mecanismos de acción y las técnicas para evaluar su efectividad. La acción antimicrobiana de los AEs se atribuye a su capacidad para penetrar las membranas celulares e inhibir las propiedades funcionales de las células fúngicas.

PALABRAS CLAVE

Hidrodestilación, metabolitos secundarios, estudios *in vitro* e *in vivo*, técnicas de contacto directo y fase de vapor, recubrimientos y películas bioactivas

INTRODUCTION

Phytopathogenic fungi are responsible for diseases in a large number of vegetable and fruit products, which may appear during crop development or postharvest (Mamgain et al. 2013). There are several methods considered to minimize losses and maintain the quality and safety of the horticultural product, being the application of chemical treatments one of the most widely used (Ruiz et al. 2012). Still, mismanagement and the presence of residues cause human health to be affected as well as resistant strains to be generated (McCarroll et al. 2002).

The purpose of the present review is to present an in-depth analysis of the information that is available on the aforementioned subject, to position the use and effectiveness of Essential oils (EOs) as a state-of-the-art alternative to control phytopathogenic fungi. In this study, EOs from 54 plant species were found to exhibit a potential role as a fungicide on 60 different fungal species. Also, this information will serve as the basis for selecting the most effective EOs for future applications into biodegradable systems in various horticultural commodities.

EOs are volatile plant derivatives (Franz and Novak 2009; Krisch et al. 2011), which are responsible for their aroma. In the plant, they serve several purposes: as messengers, attractors of pollinators, and as a defense against herbivores and microorganisms that cause diseases. This last characteristic is what confers its properties as an agent for the control of phytopathogenic fungi (Baser and Buchbauer 2009; Kubeczka 2009).

Since the Middle Ages, EOs have been used by man, not only as flavor and food preservatives, but also as cosmetics, aphrodisiacs, and medicine (Dima and Dima 2015; Mandal and Mandal 2015), as well as for the protection and control of pests and diseases in stored grains (Isman 2000). For example, a study published in 1880 describes the activity of cinnamon essential oil for the control of anthrax bacilli (Kocić-Tanackov and Dimić 2013).

Currently, there are about 2,000 species of plants from which EOs have been obtained, and around 300 of them are commercially available (Raut and Karuppayil 2014). The EOs can be obtained from various parts of the plant (Krisch et al. 2011). For

example, from the aerial part, chia (*Salvia hispanica* L.) (Elshafie et al. 2018), mint (*Mentha spicata* L.) (Kedia et al. 2015), and rue (*Ruta graveolens* L.) (Haddouchi et al. 2013); from leaves, basil (*Ocimum selloi* Benth.) (Costa et al. 2015), and orange (*Citrus sinensis* (L.) Osbeck.) (Hamdani and Allem 2015); from flowers, chamomile (*Matricaria chamomilla* L.) (Lu et al. 2013), and gypsy grass (*Bupleurum falcatum* L.) (Mohammadi et al. 2014); from bark, cinnamon (*Cinnamomum zeylanicum* Blume.) (Black-Solis et al. 2017); from the root, santolina (*Santolina chamaecyparissus* L.) (Salah-Fatnassi et al. 2017); from the rhizome, white ginger (*Hedychium coronarium* J. Koenig.) (Ray et al. 2018); from fruits, hemlock (*Cicuta virosa* var. *latisecta* Čelak.) (Tian et al. 2011a), and from seeds, dill (*Anethum graveolens* L.) (Ma et al. 2015a) and cumin (*Cuminum cyminum* L.) (Kedia et al. 2014b).

Conventionally, EOs can be obtained by two processes: cold pressure and distillation. There are several methods in connection to the latter, like hydro-distillation, and steam distillation (which can be used with high pressure) (Kubeczka 2009). EOs can also be obtained through other methods, like extraction with organic solvents, with liquid carbon dioxide, with supercritical fluids, and with microwaves (Ma et al. 2015b; Mansoreh et al. 2015; Pavlič et al. 2015; Torrenegra et al. 2015).

The content of components and the effectiveness of EOs in the control of phytopathogenic fungi depend on several factors, including the part of the plant used to obtain the EO, its concentration, as well as the environmental conditions and stress to which the botanical species was subjected during their development (Schmidt 2009), among other factors.

CONSTITUENT COMPONENTS OF EOs

Plants produce a series of secondary metabolites (more than 100,000 identified) classified into nitrogen-free compounds (terpenes, polyketides, phenolics, saponins, and polyacetylenes), and nitrogen-containing compounds (alkaloids, amines, cyanogenic glycosides, non-protein amino acids, glucosinolates, alkamides, and peptides) (Wink 2010).

Many components that constitute EOs and which are mixtures of low molecular weight compounds (between 85 and 99%) are volatile.

The main constituents are derived from three routes of biosynthesis: the mono and diterpenes from the methyl-erythritol pathway, the sesquiterpenes from the mevalonate pathway, and the phenylpropenes from the shikimic acid pathway (Franz and Novak 2009).

Terpenes are the combinations of isoprene molecules (5 carbon unit -C₅), monoterpenes (C₁₀), diterpenes (C₂₀), and sesquiterpenes (C₁₅). Terpenoids are terpenes that contain oxygen in their structure. Phenylpropane derivatives are aromatic compounds, such as aldehydes (citral), alcohols (geraniol), and phenols (thymol) (Sánchez-González et al. 2011).

In almost all the studies carried out, the identification and quantification of the constitutive components of EOs were made utilizing the gas chromatography technique coupled to mass spectrometry (GC-MS). Another method which is used with a lower frequency is the thin layer chromatography (Gemedá et al. 2015).

An EO can be constituted by more than 100 different components and in differing proportions, which can vary depending on the effect of the species (Dima and Dima 2015). There are plant species in which the main component amounted to more than 80% of the EO (Table 1). For example, in spice cloves (*Eugenia caryophyllus*, Spreng) (Bullock & S.G.Harrison), eugenol represents 90.5% (Castro et al. 2017); for basil (*O. selloi*), methyl chavicol constitutes 93.2% of the total (Costa et al. 2015); in orange (*C. sinensis*), DL-limonene, 90.6% (Singh et al. 2010); in pulicaria (*Pulicaria mauritanica* Batt.), carvotanacetone, 87.3% (Znini et al. 2013); in oregano (*Origanum vulgare* L.), carvacrol, 86.9% (Zabka et al. 2014); in mountain rue (*Ruta angustifolia* Pers.), 2-undecanone, 82.4% (Haddouchi et al. 2013); in clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry.), eugenol, 82.3% (Manganyi et al. 2015), and in cinnamon (*C. zeylanicum*) trans-cinnamaldehyde represented 80.4% (Lu et al. 2013).

Table 1. Plant species, main compounds identified and relative percentage, of EOs used for *in vitro* and *in vivo* evaluations against phytopathogenic fungi.

| Plant species | EO main compounds | Relative percentage | Reference |
|--|--|---|----------------------|
| <i>Solidago canadensis</i> L. | Germacrene D, limonene | 34.9, 12.5 | Elshafie et al. 2019 |
| <i>Helichrysum microphyllum</i> subsp. <i>tyrrhenicum</i> Bacch. & al. | Neryl acetate, c-curcumene, farnesene, 5-eudesmen-11-ol | 33.6, 11.5, 7.3, 4.3 | Juliano et al. 2019 |
| <i>Salvia officinalis</i> L. | 1,8-Cineole, α -thujone, camphor | 14.6, 20.6, 19.3 | Rguez et al. 2019 |
| <i>Rabdosia rugosus</i> Wall. (Syn. <i>Plectranthus rugosus</i> Wall.) | α -bisabolol | 41.9 | Singh et al. 2019 |
| <i>Salvia hispanica</i> L. | (Z)-caryophyllene, (E)-caryophyllene | 11.5, 10.6 | Elshafie et al. 2018 |
| <i>Hedychium coronarium</i> J. Koenig. | β -pinene, eucalyptol, linalool, coronarin-E, α -pinene, α -cymene | 12.7-42.7, 7.3-42.2, 1.9-45.1, 1.0-39.5, 3.8-16.6, 1.2-13.3 | Ray et al. 2018 |
| <i>Melaleuca alternifolia</i> (Maiden & Betche) Cheel. | Terpinene-4-ol, γ -terpinene, α -terpinene | 31.1, 25.3, 12.7 | Zhang et al. 2018 |
| <i>Cinnamomum zeylanicum</i> Blume. | Eugenol | 80.0 | Castro et al. 2017 |
| <i>Cymbopogon flexuosus</i> (Nees ex Steud.) W.Watson. | E-Caryophyllene, geraniol, geraniol, neral | 21.4, 15.3, 14.5, 14.1 | |
| <i>Eucalyptus globulus</i> Labill. | 1,8-cineole | 78.9 | |
| <i>Eugenia caryophyllus</i> (Spreng.) Bullock & S.G.Harrison. | Eugenol | 90.5 | |
| <i>Rosmarinus officinalis</i> L. | α -pinene, 1,8-cineole, camphor | 24.5, 19.4, 22.0 | |
| <i>Helichrysum italicum</i> (Roth) G.Don. | α -cedrene, α -curcumene, geranyl acetate | 13.6, 11.4, 10.0 | Djihane et al. 2017 |

| Plant species | EO main compounds | Relative percentage | Reference |
|---|---|---|-----------------------------|
| <i>Ocimum selloi</i> Benth. | Methyl chavicol | 93.2 | Costa et al. 2015 |
| <i>Trachyspermum copticum</i> (L.) Link. (Syn. <i>Trachyspermum ammi</i> (L.) Sprague.) | Thymol, α -cimene, γ -terpinene | 29.6, 16.0, 17.8 | Khosravi et al. 2015 |
| <i>Anethum graveolens</i> L. | Limonene, carvone, apiol | 32.6, 41.5, 16.7 | Ma et al. 2015a |
| <i>Thymus vulgaris</i> L. | Thymol, terpinen-4-ol | 45.2, 12.3 | Manganyi et al. 2015 |
| <i>Lippia rehmannii</i> H.Pearson. | Geranial, neral | 34.5, 20.2 | |
| <i>Syzygium aromaticum</i> (L.) Merr. & L.M.Perry. | Eugenol, β -Caryophyllene | 82.3, 11.0 | |
| <i>Cinnamomum zeylanicum</i> | Eugenol | 12.8 | |
| <i>Thymus vulgaris</i> | α -cimene, thymol | 21.9, 44.6 | Matusinsky et al. 2015 |
| <i>Pelargonium odoratissimum</i> (L.) L'Hér. | β -citronellol, geraniol | 24.8, 12.5 | |
| <i>Thymus vulgaris</i> | Thymol, α -cimene, γ -terpinene | 35.6, 19.5, 10.5 | Pekmezovic et al. 2015 |
| <i>Cinnamomum cassia</i> L. | Cinnamaldehyde, cinnamyl acetate | 73.6, 3 - 15 | |
| <i>Anthemis odontostephana</i> Boiss. Var. <i>odontostephana</i> | Flower: borneol, (-)-bornyl acetate Leaf: borneol, myristicin Stem: borneol, myristicin | Flor: 31.3, 13.9 Hoja: 19.2, 13.3 Tallo: 27.0, 11.2 | Zebarjad and Farjam 2015 |
| <i>Artemisia monosperma</i> Delile. | Capylene, capilin, γ -terpinene | 36.8, 14.6, 12.4 | Badawy and Abdelgaleil 2014 |
| <i>Cymbopogon nardus</i> (L.) Rendle. | Citronellal, citronellol, geraniol | 26.2, 12.9, 19.7 | Chen et al. 2014 |
| <i>Mentha spicata</i> L. | DL-Limonene, dextro-carvone | 25.5, 59.6 | Kedia et al. 2014a |
| <i>Cuminum cyminum</i> L. | Laevo β -pinene, cimeno, γ -terpinene, cuminaldehyde | 11.5, 47.0, 19.3, 14.9 | Kedia et al. 2014b |
| <i>Thymus kotschyanus</i> Boiss. & Hohen. | Thymol | 46.7 | Mohammadi et al. 2014 |
| <i>Thymus daenensis</i> Celak. | α -terpineol, thymol, carvacrol | 22.9, 20.2, 31.4 | |
| <i>Stachys pubescens</i> Ten. | 2,6-octadiene, δ -cadinene, germacrene | 11.5, 19.7, 22.4 39.1, 19.6 | |
| <i>Bupleurum falcatum</i> L. | Torilenol, spatulenol | | |
| <i>Satureja hortensis</i> L. | Carvacrol, α -cimene | 50.0, 12.2 | Stević et al. 2014 |
| <i>Origanum heracleoticum</i> L. | Carvacrol | 75.8 | |
| <i>Thymus vulgaris</i> | Thymol, α -cimene | 43.7, 23.2 | |
| <i>Rosa \times damascena</i> Herrm. | Citronellol, geraniol | 51.3, 25.8 | |
| <i>Pelargonium graveolens</i> L'Hér. | Citronellol, geraniol | 39.0, 11.3 | |
| <i>Melaleuca alternifolia</i> | Terpinen-4-ol, γ -terpinene | 40.7, 17.8 | |
| <i>Laurus nobilis</i> L. | Cinnamaldehyde, eugenol | 30.2, 44.1 | Xu et al. 2014 |
| <i>Carum carvi</i> L. | Carvone, limonene | 68.7, 21.5 | Zabka et al. 2014 |
| <i>Coriandrum sativum</i> L. | Linalool | 66.7 | |
| <i>Mentha arvensis</i> L. | Menthol, menthon, | 42.1, 21.7, 13.6 | |
| <i>Ocimum citriodorum</i> Vis. | isomenthon | 31.1, 23.6, 16.4 | |
| <i>Origanum vulgare</i> L. | Geranial, neral, linalool | 86.9 | |
| <i>Pimenta racemosa</i> (Mill.) J.W.Moore. | Carvacrol | 64.0, 14.6 | |
| <i>Thymus satureoides</i> Coss. & Balansa. | Eugenol, myrcen | 29.8, 12.0, 10.0 | |
| <i>Thymus vulgaris</i> | Borneol, thymol, carvacrol | 60.2, 19.9 | |
| <i>Thymus vulgaris</i> | Thymol, α -cimene | | |

| Plant species | EO main compounds | Relative percentage | Reference |
|--|--|------------------------|----------------------------|
| <i>Ruta angustifolia</i> Pers. | 2-decanone, 2-undecanone | 10.0, 82.4 | Haddouchi et al. 2013 |
| <i>Ruta chalepensis</i> var. <i>bracteosa</i> (DC.) Boiss. | 2-nonanone, 1-nonene, 2-undecanone | 32.7, 13.9, 32.5 | |
| <i>Ruta graveolens</i> L. | 2-nonanone, 2-undecanone | 21.6, 55.4 | |
| <i>Cinnamomum zeylanicum</i> | 3-phenylpropionaldehyde, <i>trans</i> -cinnamaldehyde | 10.4, 80.4 | Lu et al. 2013 |
| <i>Pelargonium graveolens</i> | Citronellol, citronellyl formate | 41.5, 10.8 | |
| <i>Cuminum cyminum</i> | β -pinene, α -cymene, γ -terpinene, cuminaldehyde | 14.0, 16.8, 11.3, 46.9 | |
| | α -cymene, thymol | | |
| <i>Thymus vulgaris</i> | Linalool, methyl chavicol | 37.6, 39.7 | |
| <i>Ocimum basilicum</i> L. | Citronellal, citronellol, | 45.9, 35.0 | |
| <i>Cymbopogon citratus</i> (DC.) Stapf. | geranial | 20.7, 14.1, 26.0 | |
| <i>Melaleuca alternifolia</i> | Terpinen-4-ol | 50.2 | Shao et al. 2013 |
| <i>Pulicaria mauritanica</i> Batt. | Carvotanacetone | 87.3 | Znini et al. 2013 |
| <i>Eucalyptus tereticornis</i> Sm. | α -pinene, β -pinene, 1,8-cineole | 22.5, 22.5, 19.8 | Guleria et al. 2012 |
| <i>Armoracia rusticana</i> P.Gaertn., B.Mey. & Scherb. | Allyl isothiocyanate, β -phenylethyl isothiocyanate | 63.7, 23.9 | Kloucek et al. 2012 |
| | Diallyl disulfide, diallyl trisulfide | 43.8, 27.1 79.3 | |
| <i>Allium sativum</i> L. | <i>trans</i> -cinnamaldehyde | 28.4, 20.2, 17.9 | |
| <i>Cinnamomum aromaticum</i> Nees. | Carvacrol, thymol, α -cymene | | |
| <i>Origanum compactum</i> Benth. | | | |
| <i>Zataria multiflora</i> Boiss. | Carvacrol | 76.1 | Mahmoudi et al. 2012 |
| <i>Cicuta virosa</i> var. <i>latisecta</i> Celak. | α -cymene, γ -terpinene, cuminaldehyde | 27.9, 40.9, 21.2 | Tian et al. 2011a |
| <i>Thymus vulgaris</i> | γ -terpinene, α -cymene | 17.1, 14.6 | Bosquez-Molina et al. 2010 |
| <i>Citrus aurantifolia</i> (Christm.) Swingle. | <i>D</i> -Limonene, β -pinene, γ -terpinene | 45.1, 20.5, 10.5 | |
| <i>Verbena officinalis</i> L. | Isobornyl formate, (<i>E</i>)-citral | 45.4, 44.5 | Camele et al. 2010 |
| <i>Thymus vulgaris</i> | α -cymene, carvacrol | 56.2, 24.4 | |
| <i>Origanum vulgare</i> | α -cymene, carvacrol | 41.9, 44.0 | |
| <i>Citrus maxima</i> (Burm.) Merr. | <i>DL</i> -Limonene, 1-hexene, 4-Methy, <i>Z</i> -citral, <i>E</i> -citral | 31.8, 15.2, 13.3, 17.7 | Singh et al. 2010 |
| <i>Citrus sinensis</i> (L.) Osbeck. | <i>DL</i> -Limonene | 90.66 | |

Some compounds are present in several plant species in different concentrations. For example, carvacrol is present in four species: oregano (*O. vulgare*), 44.0% (Camele et al. 2010); *Zataria* (*Zataria multiflora* Boiss.), 76.1% (Mahmoudi et al. 2012); summer savory (*Satureja hortensis* L.), 50.0% (Stević et al. 2014), and avishan-e-denaii (*Thymus daenensis* Celak.), 31.4% (Mohammadi et al. 2014); eugenol, in four species: clove (*S. aromaticum*), 82.3%; cinnamon (*C. zeylanicum*), 12.8% (Manganyi et al. 2015); sweet

bay (*Laurus nobilis* L.), 44.1% (Xu et al. 2014); and bay rum tree (*Pimenta racemosa* (Mill.) J.W.Moore.), 64.0% (Zabka et al. 2014); the geranial in three species: *lipidia* (*Lippia rehmannii* H.Pearson.), 34.5% (Manganyi et al. 2015); basil sweet-lemon (*Ocimum citriodorum* Vis.), 31.1% (Zabka et al. 2014), and lemongrass (*Cymbopogon citratus* (DC.) Stapf.), 26.0% (Lu et al. 2013); finally, linalool, in two species: coriander (*Coriandrum sativum* L.), 66.7% (Zabka et al. 2014), and sweet basil (*Ocimum basilicum* L.), 45.9% (Lu et al. 2013). These compounds

are secondary metabolites that occur in some plant species and serve as a defense against microorganisms. According to Sell (2009), few of them share the same biosynthetic pathways.

In other cases, the same component can vary in concentration between species of the same genus. The 2-undecanone level is 82.4% in mountain rue (*R. angustifolia*), 55.4% in rue (*R. graveolens*), and 32.5% in fringed rue (*Ruta chalepensis* var. *bracteosa* [DC.] Boiss.) (Haddouchi et al. 2013). Carvacrol represents 86.9% in oregano (*O. vulgare*) (Zabka et al. 2014), 75.8% in oregano (*O. heracleoticum* L.) (Stević et al. 2014), and 28.4% in sahtar (*O. compactum* Benth.) (Kloucek et al. 2012), whereas thymol can be quantified as 60.2% in thyme (*T. vulgaris* L.) (Zabka et al. 2014), but it can be 46.7% in other species such as *T. kotschyanus* Boiss. & Hohen. (Mohammadi et al. 2014).

There are also variations in the concentration of the same compound in the same species, for example, thymol in *T. vulgaris*, with 35.6 to 60.2% (Pekmezovic et al. 2015; Zabka et al. 2014), carvacrol in *O. vulgare*, 44.0 to 86.9% (Camele et al. 2010; Zabka et al. 2014), and terpinen-4-ol in tea tree (*Melaleuca alternifolia* [Maiden & Betche] Cheel.), with 31.1 to 50.2% (Shao et al. 2013; Stević et al. 2014; Zhang et al. 2018). According to Schmidt (2009), the content of EOs components may depend on several factors, including the organ of the plant used, environmental conditions, and the stress to which the botanical species was subjected during its development in the field.

POTENTIAL OF EOS FOR CONTROLLING PHYTOPATHOGENIC FUNGI

EOs have been tested for the control of phytopathogenic fungi in *in vitro* and *in vivo* studies. The literature reports that the effectiveness of EOs varied depending on the plant species, microorganism, EO concentration, and the evaluation technique used, among other factors.

TECHNIQUES TO EVALUATE THE EFFECTIVENESS OF THE EOS ON FUNGAL DEVELOPMENT

Two techniques are used to evaluate the effectiveness of EOs. The first is through direct contact, in which case, the EO is mixed with the culture medium, either

solid or liquid (*in vitro* studies), or placed directly on the fruit (*in vivo* studies). The second is through vapor phase, where the EO is impregnated in an absorbent element, and then it is volatilized flooding the environment where the fungus has been placed (Petri dish for *in vitro* studies and hermetic containers for *in vivo* studies).

Thus, the same technique can have different names. When it involves direct contact in the solid medium it is known as toxic medium assay (Manganyi et al. 2015), poisoned medium technique (Chen et al. 2014), dilution in agar method (Hamdani and Allem 2015), radial growth inhibition technique (Badawy and Abdelgaleil 2014), and agar well diffusion method (Ray et al. 2018). Other methods reported *in vitro* are direct contact in the liquid medium, micro, and macro dilution in broth (Juliano et al. 2019; Khosravi et al. 2015; Martins et al. 2013), and in the vapor phase, disc diffusion (Kloucek et al. 2012; Rguez et al. 2019; Zhang et al. 2018), drop diffusion test (Saroj et al. 2015), and soaked cotton (Mahmoudi et al. 2012).

In *in vivo* studies, the following techniques have been reported: by direct contact and aspersion (Camele et al. 2010), by immersion (Bosquez-Molina et al. 2010), in the vapor phase, soaked cotton (Kedia et al. 2014b), sterile paper discs (Stević et al. 2014), and drop (Mehra et al. 2013).

To evaluate the effectiveness of EOs, we used the minimum inhibitory concentration (MIC), which is defined as the lowest EO concentration that completely inhibits the growth of the phytopathogenic fungus —a synonym used for this is ‘the minimum inhibitory amount’ (MIA) (Guleria et al. 2012, Van de Vel et al. 2019). The minimum fungicidal concentration (MFC), defined as the lowest concentration, resulted in zero fungus growth when it is cultivated again in a culture medium —and a synonym for it could be ‘the minimum lethal concentration’ (MLC) (Martins et al. 2013).

EOS IN THE CONTROL OF PHYTOPATHOGENIC FUNGI IN VITRO

Table 2 presents a series of studies in which EOs obtained from 54 plant species were effective in controlling 60 species of phytopathogenic fungi. The genera with the highest number of species were: *Citrus* (Bosquez-Molina et al. 2010; Dimić et al. 2014;

Singh et al. 2010), and *Ruta*, both from the Rutaceae family (Haddouchi et al. 2013) with four species each, the genera *Cinnamomum* (Lauraceae family) (Kloucek et al. 2012; Manganyi et al. 2015; Pekmezovic et al. 2015), *Ocimum* (Costa et al. 2015; Lu et al. 2013; Zabka et al. 2014), and *Thymus* (Lamiaceae family) (Camele et al. 2010; Mohammadi et al. 2014; Zabka et al. 2014), with three species each, and the genera *Pelargonium* (Geraniaceae family) (Lu et al. 2013; Matusinsky et al. 2015), *Mentha* (Kedia et al. 2014a; Lu et al. 2013), *Origanum* (Lamiaceae family) (Stević et al. 2014; Zabka et al. 2014), *Helichrysum* (Asteraceae family) (Juliano et al. 2019; Ray et al. 2018), and *Salvia* (Lamiaceae family) (Elshafie et al. 2018; Rguez et al. 2019), with two species each.

Botanical species with the greatest capacity reported for *in vitro* fungi control were dill (*Apium graveolens* L.) against *Sclerotinia sclerotiorum* (Lib.) de Bary. in 0.12 $\mu\text{L mL}^{-1}$ doses (Ma et al. 2015a); oregano (*O. vulgare*) against *Aspergillus flavus* Link., and *A. niger* P.E.L. van Tieghem. (de Sousa et al. 2013), and verbena (*Verbena officinalis* L.), thyme (*T. vulgaris*), and oregano (*O. vulgare*) for *Botrytis cinerea* Pers., *Penicillium italicum* Wehmer., and *Phytophthora citrophthora* R.&E. Sm. Control (0.25 $\mu\text{L mL}^{-1}$) (Camele et al. 2010).

They are followed by tea tree EOs (*M. alternifolia*) against *B. cinerea*, 0.4 $\mu\text{L mL}^{-1}$ dose (Shao et al. 2013), basil (*O. selloi*) against *Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora. (Costa et al. 2015), thyme (*T. vulgaris*), clove (*S. aromaticum*), and cinnamon (*C. zeylanicum*) against *Fusarium oxysporum* Schlecht. (Manganyi et al. 2015), and everlasting (*Helichrysum splendidum* [Thunb.] Less.) against *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in 0.5 $\mu\text{L mL}^{-1}$ doses (Mashigo et al. 2015). The EO of cumin (*C. cyminum*) against *A. flavus*, 0.6 $\mu\text{L mL}^{-1}$ doses (Kedia et al. 2014b), grapefruit (*Citrus maxima* (Burm.) Merr.), and orange (*C. sinensis*) against the same microorganism in 0.75 $\mu\text{L mL}^{-1}$ doses (Singh et al. 2010), and Mexican lemon (*C. aurantifolia* (Christm.) Swingle.), and thyme (*T. vulgaris*) against *C. gloeosporioides* in 0.85 $\mu\text{L mL}^{-1}$ doses (Bosquez-Molina et al. 2010).

Finally, effective EOs that are reported at concentrations of less than 1 $\mu\text{L mL}^{-1}$ were peppermint (*M. spicata*) against *A. flavus* (Kedia et al. 2014a), rosemary (*Rosmarinus officinalis* L.) against *A. flavus* and *A. niger* (de Sousa et al. 2013), mint geranium (*Pelargonium odo-*

ratissimum (L.) L'Hér.), and thyme (*T. vulgaris*) against *Oculimacula yallundae* (Walker & Spooner) Crous & Gams., *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous., and *Pyrenophora teres* Drechsler. (Matusinsky et al. 2015), and caraway (*Carum carvi* L.), lemon basil (*O. citriodorum*), oregano (*O. vulgare*), malagueta (*P. tolonif*), Moroccan thyme (*Thymus saturooides* Coss. & Balansa.), and thyme (*T. vulgaris*) against *A. niger*, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries., and *Stachybotrys chartarum* (Ehrenb.) S. Hughes. (Zabka et al. 2014).

EOs IN THE CONTROL OF PHYTOPATHOGENIC FUNGI *IN VIVO*

Overall, the reported EO with the lowest effective dose (0.50 $\mu\text{L mL}^{-1}$) was dill (*A. graveolens*) for the control of white rot (*S. sclerotiorum*) in artificially inoculated leaves of turnip (*Brassica napus* L.). When the EO was sprayed before inoculation, the development of the pathogen was inhibited. At the same time, in the control treatment, lesions reached up to 30.2 mm in diameter (Ma et al. 2015a) (Table 2).

In the vapor phase, the cumin EO (*C. cyminum*) with a 0.6 $\mu\text{L mL}^{-1}$ dose, for the control of *A. flavus* in wheat seeds (*Triticum aestivum* L.) and chickpea (*Cicer arietinum* L.), with about 65.1% and 50.0%, respectively after 12 months of storage (Kedia et al. 2014b). Likewise, *A. flavus* and *A. niger* fungi of the table grapes (*Vitis labrusca* L.) were inhibited for 12 and 24 days, by immersion in rosemary EO (*R. officinalis*) at 1 $\mu\text{L mL}^{-1}$ concentration when stored at room temperature (25°C) and 12°C, respectively (de Sousa et al. 2013).

In papaya fruit (*Carica papaya* L.), thyme EO (*T. vulgaris*) was effective for the control of anthracnose disease (*C. gloeosporioides*) employing an immersion treatment of 1.5 $\mu\text{L mL}^{-1}$. When the EO was applied after inoculation, the inhibition was 100%. In contrast, if the treatment was applied after inoculation, a 50% inhibition was achieved (Bosquez-Molina et al. 2010).

The application by sprinkling of 2 $\mu\text{L mL}^{-1}$ of thyme EO (*T. vulgaris*) in orange fruits (*C. sinensis*) for controlling gray mold (*B. cinerea*), phytophthora (*P. citrophthora*), and rhizopus rot (*Rhizopus tolonifera* (Ehrenb.) Vuill.) was 100% effective (Camele et al. 2010).

Table 2. Plant species and EOs doses applied for *in vitro* and *in vivo* control of various phytopathogenic fungi.

| Plant species | Phytopathogenic fungi | Evaluation | EO dose | Reference |
|--|---|--|---|--------------------------|
| <i>Solidago canadensis</i> | <i>Monilinia fructicola</i> (G. Winter) Honey. | <i>In vitro</i> | 1000 µg mL ⁻¹ | Elshafie et al. 2019 |
| <i>Helichrysum microphyllum</i> subsp. <i>tyrrhenicum</i> | <i>Aspergillus brasiliensis</i> Varga, Frisvad & Samson | <i>In vitro</i> | 2.00 mg mL ⁻¹ | Juliano et al. 2019 |
| <i>Salvia officinalis</i> | <i>Botrytis cinerea</i> Pers., <i>Fusarium sambucinum</i> Fuckel. | <i>In vitro</i> | 1.93 µg mL ⁻¹ | Rguez et al. 2019 |
| <i>Rabdosia rugosus</i> | <i>Rhizoctonia solani</i> J.G. Kühn., <i>Sclerotium rolfsii</i> Sacc., <i>Fusarium oxysporum</i> Schlecht. | <i>In vitro</i> | 250 ppm | Singh et al. 2019 |
| <i>Salvia hispanica</i> | <i>Monilinia laxa</i> (Aderh. y Ruhl.), <i>M. fructicola</i> , <i>M. fructigena</i> (Aderh. & Ruhland) Honey., <i>Aspergillus fumigatus</i> Fresen., <i>Penicillium digitatum</i> (Pers.) Sacc., <i>P. expansum</i> Link. | <i>In vitro</i> | 40% | Elshafie et al. 2018 |
| <i>Hedychium coronarium</i> | <i>Aspergillus niger</i> P.E.L., <i>A. flavus</i> Link., <i>Fusarium oxysporum</i> | <i>In vitro</i> | 6.25 µg mL ⁻¹ | Ray et al. 2018 |
| <i>Melaleuca alternifolia</i> | <i>Penicillium italicum</i> Wehmer., <i>P. digitatum</i> | <i>In vitro</i> | 12 mg mL ⁻¹ | Zhang et al. 2018 |
| <i>Helichrysum italicum</i> | <i>Fusarium solani</i> var. <i>coeruleum</i> (Lib. ex Sacc.) C. Booth., <i>Aspergillus niger</i> , <i>Ascochyta rabiei</i> (Pass.) Labr. | <i>In vitro</i> | 50.6 µg mL ⁻¹ | Djihane et al. 2017 |
| <i>Ocimum selloi</i> | <i>Moniliophthora perniciosa</i> (Stahel) Aime & Phillips-Mora. | <i>In vitro</i> | 500 ppm | Costa et al. 2015 |
| <i>Lippia adoensis</i> var. <i>koseret</i> Sebsebe. | <i>Aspergillus flavus</i> , <i>A. parasiticus</i> Speare., <i>A. niger</i> , <i>A. fumigatus</i> | <i>In vitro</i> | 2 µL mL ⁻¹ | Gemeda et al. 2015 |
| <i>Anethum graveolens</i> | <i>Sclerotinia sclerotiorum</i> (Lib.) de Bary. | <i>In vitro</i> <i>In vivo</i> (turnip) | Medium - 1.0 µL mL ⁻¹ Air - 0.125 µL mL ⁻¹ 0.50 µL mL ⁻¹ | Ma et al. 2015a |
| <i>Thymus vulgaris</i> <i>Syzygium aromaticum</i> <i>Cinnamomum zeylanicum</i> | <i>Fusarium oxysporum</i> | <i>In vitro</i> | 500 µL L ⁻¹ | Manganyi et al. 2015 |
| <i>Helichrysum splendidum</i> (Thunb.) Less. | <i>Colletotrichum gloeosporioides</i> (Penz.) Penz. & Sacc. | <i>In vitro</i> | 500 µL L ⁻¹ | Mashigo et al. 2015 |
| <i>Thymus vulgaris</i> <i>Pelargonium odoratissimum</i> | <i>Oculimacula yallundae</i> (Walker & Spooner) Crous & Gams. (Desm.) Quaedvlieg & Crous. <i>Pyrenophora teres</i> Drechsler. | <i>In vitro</i> | 1.0 µL mL ⁻¹ | Matusinsky et al. 2015 |
| <i>Thymus vulgaris</i> <i>Cinnamomum cassia</i> | <i>Aspergillus flavus</i> | <i>In vitro</i> | 31.2 µg mL ⁻¹ 62.5 µg mL ⁻¹ | Pekmezovic et al. 2015 |
| <i>Anthemis odontostephana</i> var. <i>odontostephana</i> | <i>Aspergillus niger</i> <i>Fusarium solani</i> (Mart.) Sacc. | <i>In vitro</i> | 32.6 µg mL ⁻¹ | Zebarjad and Farjam 2015 |

| Plant species | Phytopathogenic fungi | Evaluation | EO dose | Reference |
|---|--|--|--------------------------------|-----------------------|
| <i>Citrus limon</i> (L.) Osbeck. | <i>Aspergillus parasiticus</i> , <i>A. carbonarius</i> (Bainier) Thom. <i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries. <i>Eurotium herbariorum</i> (F.H. Wigg.) Link. <i>Penicillium chrysogenum</i> Thom. | <i>In vitro</i> | 2.5 $\mu\text{L mL}^{-1}$ | Dimić et al. 2014 |
| <i>Mentha spicata</i> | <i>Absidia ramosa</i> (Zopf) Lendn. <i>Aspergillus flavus</i> , <i>A. fumigatus</i> , <i>A. glaucus</i> (L.) Link., <i>A. niger</i> , <i>A. unguis</i> (Émile-Weill & L. Gaudin) Dodge. <i>Cladosporium cladosporioides</i> <i>Curvularia lunata</i> (Wakker) Boedijn. <i>Fusarium oxysporum</i> <i>Mycelia sterilia</i> <i>Penicillium citrinum</i> Thom., <i>P. italicum</i> , <i>P. luteum</i> Zukal., <i>P. purpurogenum</i> Stoll. <i>Rhizopus stolonifer</i> (Ehrenb.) Vuill. <i>Spondylocladium australe</i> J.C. Gilman & E.V. Abbott. | <i>In vitro</i> | 1.0 $\mu\text{L mL}^{-1}$ | Kedia et al. 2014a |
| <i>Cuminum cyminum</i> | <i>Absidia ramosa</i> <i>Aspergillus flavus</i> , <i>A. fumigatus</i> , <i>A. glaucus</i> , <i>A. niger</i> , <i>A. unguis</i> <i>Cladosporium cladosporioides</i> <i>Curvularia lunata</i> <i>Fusarium oxysporum</i> <i>Mycelia sterilia</i> <i>Penicillium citrinum</i> , <i>P. italicum</i> , <i>P. luteum</i> , <i>P. purpurogenum</i> <i>Rhizopus stolonifer</i> <i>Spondylocladium australe</i> | <i>In vitro</i> <i>In vivo</i> (wheat and chickpea) | 0.6 $\mu\text{L mL}^{-1}$ | Kedia et al. 2014b |
| <i>Thymus kotschyanus</i> <i>Stachys pubescens</i> | <i>Fusarium oxysporum</i> <i>Aspergillus flavus</i> | <i>In vitro</i> | 0.5 $\mu\text{g mL}^{-1}$ | Mohammadi et al. 2014 |
| <i>Pinus nigra</i> subsp. <i>pallasiana</i> (Lamb.) Holmboe. <i>Pinus nigra</i> var. <i>banatica</i> Georgescu & Ionescu | <i>Aspergillus niger</i> | <i>In vitro</i> | 5 mg mL^{-1} | Šarac et al. 2014 |
| <i>Satureja hortensis</i> <i>Origanum heracleoticum</i> <i>Thymus vulgaris</i> <i>Rosa</i> × <i>damascena</i> | <i>Fusarium subglutinans</i> (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasas., <i>F. solani</i> , <i>F. tricinctum</i> (Corda) Sacc., <i>F. sporotrichioides</i> Sherb., <i>F. verticillioides</i> (Sacc.) Nirenberg., <i>F. oxysporum</i> , <i>F. semitectum</i> Berk. & Ravenel., <i>F. equiseti</i> (Corda) Sacc. <i>Aspergillus flavus</i> , <i>A. niger</i> <i>Gliocladium roseum</i> Bainier. <i>Curvularia lunata</i> | <i>In vitro</i> | 0.07 a 0.9 mg mL^{-1} | Stević et al. 2014 |

| Plant species | Phytopathogenic fungi | Evaluation | EO dose | Reference |
|--|---|---|---|-----------------------|
| <i>Satureja hortensis</i> <i>Origanum heracleoticum</i> <i>Thymus vulgaris</i> <i>Rosa × damascena</i> | <i>Verticillium dahliae</i> Kleb. <i>Trichoderma viride</i> Pers., <i>T. roseum</i> Pers. <i>Myrothecium verrucaria</i> (Alb. & Schwein.) Ditmar. | <i>In vitro</i> | 0.07 a 0.9 mg mL ⁻¹ | Stević et al. 2014 |
| <i>Carum carvi</i> <i>Ocimum citriodorum</i> <i>Origanum vulgare</i> <i>Pimenta racemosa</i> <i>Thymus satureoides</i> <i>Thymus vulgaris</i> | <i>Stachybotrys chartarum</i> (Ehrenb.) S. Hughes. <i>Cladosporium cladosporioides</i> <i>Aspergillus niger</i> | <i>In vitro</i> | 1.0 µL mL ⁻¹ | Zabka et al. 2014 |
| <i>Origanum vulgare</i> <i>Rosmarinus officinalis</i> | <i>Aspergillus niger</i> , <i>A. flavus</i> | <i>In vitro</i> <i>In vivo</i> (grapes) | 0.25 µL mL ⁻¹ 1.0 µL mL ⁻¹ | de Sousa et al. 2013 |
| <i>Ruta angustifolia</i> <i>Ruta chalepensis</i> var. <i>bracteosa</i> <i>Ruta graveolens</i> <i>Ruta tuberculata</i> Forssk. | <i>Aspergillus fumigatus</i> , <i>A. flavus</i> <i>Cladosporium herbarum</i> (Pers.) Link. <i>Fusarium oxysporum</i> | <i>In vitro</i> | 10 µL disk | Haddouchi et al. 2013 |
| <i>Cinnamomum zeylanicum</i> <i>Thymus vulgaris</i> <i>Ocimum basilicum</i> <i>Cuminum cyminum</i> <i>Pelargonium graveolens</i> <i>Cymbopogon citratus</i> <i>Litsea cubeba</i> (Lour.) Pers. <i>Mentha arvensis</i> | <i>Colletotrichum destructivum</i> O'Gara. <i>Phytophthora parasitica</i> var. <i>nicotianae</i> (Breda de Haan) Tucker. | <i>In vitro</i> | 240 µg mL ⁻¹ | Lu et al. 2013 |
| <i>Eucalyptus globulus</i> | <i>Mucor hiemalis</i> Wehmer. <i>Penicillium glabrum</i> (Wehmer) Westling. <i>Fusarium roseum</i> Link. | <i>In vitro</i> | 1.5 a 5.0 µL mL ⁻¹ | Martins et al. 2013 |
| <i>Melaleuca alternifolia</i> | <i>Botrytis cinerea</i> | <i>In vitro</i> | 0.4 a 1.0 mL l ⁻¹ | Shao et al. 2013 |
| <i>Pulicaria mauritanica</i> | <i>Penicillium expansum</i> | <i>In vitro</i> | 2.0 µL mL ⁻¹ | Znini et al. 2013 |
| <i>Armoracia rusticana</i> <i>Allium sativum</i> <i>Cinnamomum aromaticum</i> | <i>Aspergillus niger</i> <i>Penicillium digitatum</i> | <i>In vitro</i> | 31.2 µL L ⁻¹ 62.5 µL L ⁻¹ | Kloucek et al. 2012 |
| Citri-V | <i>Penicillium chrysogenum</i> <i>Aspergillus niger</i> | <i>In vitro</i> <i>In vivo</i> (Tomato) | Air - 15 mg L ⁻¹ | Phillips et al. 2012 |
| <i>Mazus goodenifolius</i> (Hornem.) Pennell. | <i>Rhizopus solani</i> | <i>In vitro</i> | 20 mg mL ⁻¹ | Riaz et al. 2012 |
| <i>Cicuta virosa</i> var. <i>latisecta</i> | <i>Aspergillus flavus</i> , <i>A. oryzae</i> (Ahlb.) Cohn., <i>A. niger</i> | <i>In vitro</i> <i>In vivo</i> (Cherry tomato) | 5 µL mL ⁻¹ 200 µL mL ⁻¹ | Tian et al. 2011a |

| Plant species | Phytopathogenic fungi | Evaluation | EO dose | Reference |
|---|--|---|--|-----------------------------------|
| <i>Anethum graveolens</i> | <i>Aspergillus flavus</i> , <i>A. oryzae</i> , <i>A. niger</i> | <i>In vitro</i> <i>In vivo</i> (Cherry tomato) | 2.0 $\mu\text{L mL}^{-1}$ 120 $\mu\text{L mL}^{-1}$ | Tian et al. 2011b |
| <i>Thymus vulgaris</i> <i>Citrus aurantifolia</i> | <i>Colletotrichum gloeosporioides</i> | <i>In vitro</i> <i>In vivo</i> (papaya) | 0.085 % (v/w) 0.15% (v/w) | Bosquez- Molina et al. 2010 |
| <i>Origanum vulgare</i> <i>Verbena officinalis</i> <i>Thymus vulgaris</i> | <i>Botrytis cinerea</i> <i>Penicillium italicum</i> <i>Phytophthora citrophthora</i> R.&E. Sm. <i>Rhizopus stolonifer</i> | <i>In vitro</i> <i>In vivo</i> (orange) | 250 ppm 2000 ppm | Camele et al. 2010 |
| <i>Citrus maxima</i> <i>Citrus sinensis</i> | <i>Aspergillus flavus</i> | <i>In vitro</i> | 750 ppm | Singh et al. 2010 |

Does not include the species *Alternaria alternata*. Citri-V = 50:50 mix of orange and bergamot essential oil.

MECHANISMS OF ACTION OF EOs ON PHYTOPATHOGENIC FUNGI

The action mechanism of the EOs on fungi is not entirely known. However, Rivera et al. (2015) proposed that the antimicrobial action is attributed to their ability to penetrate through the cell membranes and inhibit the functional properties of the cell by causing severe damage to organelles. There are different microscopy techniques used to observe these physical alterations. Overall, the Optical Microscopy and Scanning Electron Microscopy (SEM) are used to recognize the morphological changes in fungi. In contrast, the Transmission Electron Microscopy (TEM) is used to look into cellular alterations.

It is generally reported in the literature that, in control treatments, the fungi presented mycelium with regular morphology, healthy development, abundant conidia, and round hyphae with smooth external surfaces, constant diameter, and integral membranes. On the contrary, in EOs-treated fungi, it was possible to observe different morphological alterations and structural damage, which increased depending on the concentration.

For example, the application of the cinnamon EO (*C. zeylanicum*) in *Alternaria alternata* (Fr.) Keissl., produce alterations in its hyphae collapse, crushing, emptying, and lysis of the cell wall, and with aggregates of wrinkled hyphae (Lu et al. 2013). In other studies, thyme EO (*T. vulgaris*) affected the conidia germination

(and in some cases, the total absence of these) together with a delay in the development of the germinative tube, rupture of the cell wall and the conidia plasma membrane, with disorganization of the cytoplasm and destruction of organelles (Perina et al. 2014), while citronella EO (*Cymbopogon nardus* (L.) Rendle.) affected the mycelial morphology with empty, folded, and flattened hyphae, with a rough and deformed surface, with the absence of conidia (Chen et al. 2014).

In the same fungus, with laurel EO (*L. nobilis*), the mycelium was degraded with folds and wrinkles on the cell walls surface and few visible spores (Xu et al. 2014). Za'atar EO (*Z. multiflora*) is reported to cause the granulation of cytoplasm, inhibition of mycelial growth, decrease and loss in the conidia formation, inhibition of conidial germination and shortening of the germinative tubes, as well as, a reduction in the diameter of hyphae (with sub-apical germination and with anomalous bulbous structures at its tips), collapse and wrinkling of hyphae and production of chlamydoconidia in old hyphae (Mahmoudi et al. 2012).

In other phytopathogenic fungi, the damages observed were similar. For example, *A. flavus* incubated in peppermint EO (*M. spicata*), presented hyphae distorted with collapsed conidia and depressions in the surface. Also, through TEM observations, plasma membrane rupture, leakage of cellular material, and decrease of the cellular matrix (Kedia et al. 2015) were documented, while *A. niger* treated with dill EO (*A. graveolens*) showed deformation of the conidia, with

severe damage, rupture and loss of integrity in these structures, as well as, craters in the vesicles, aggregates of wrinkled hyphae, decrease in diameter, collapse and flattening (Tian et al. 2011b).

In *B. cinerea*, tea tree EO (*M. alternifolia*) inhibited fungal growth and spore germination, and empty, collapsed, flat hyphae also occurred. Observations by TEM showed that the cell wall was destroyed. Therefore, the cell membrane permeability increased, in addition to cytoplasm coagulation by a higher density of electrons, increased mitochondrial volume, and loss of organelles (Shao et al. 2013).

In *Colletotrichum destructivum* O’Gara., thyme EO (*T. vulgaris*) caused considerable mycelium damage as aggregates of wrinkled, collapsed, crushed, and empty hyphae, and cinnamon EO (*C. zeylanicum*) on *Phytophthora parasitica* var. *nicotianae* (Breda de Haan) Tucker. caused alterations similar to those mentioned for *C. destructivum* (Lu et al. 2013).

USE OF EOs AS COMPONENTS OF COATINGS AND BIOACTIVE FILMS FOR CONTROLLING PHYTOPATHOGENIC FUNGI

On the issue of antimicrobial substances, EOs are useful for controlling phytopathogenic fungi. However, their direct application in food brings about some problems: rapid volatility, aroma transfer, and toxicity. For this reason, its use has been studied in films and coatings, which allows them to minimize application doses; also, their gradual migration from the matrix allows their release to be scheduled (Ouattara et al. 2000; Sánchez-González et al. 2011).

On the use of biodegradable polymers for the development of coatings and edible films to which EOs were added and which were later evaluated in the control of phytopathogenic fungi (Table 3), it was found that 47.3% of the works focused on the development of coatings (Guerra et al. 2015; Vu et al. 2011), and an equal percentage on film development (47.3%) (Avila-Sosa et al. 2010; Hemalatha et al. 2017; Pola et al. 2016).

The EOs most used in the formulations were those from oregano (*O. vulgare*) (Barreto et al. 2016; Raphaël and Meimandipour 2017), and thyme (*T. vulgaris*) (Bill et al. 2014; Yahyaoui et al. 2016), followed by lemongrass (*C. citratus*) (Avila-Sosa et al. 2012; Ben-Fadhel et al. 2017), and cinnamon (*C. zeylanicum*) (Mateo et al. 2017; Noshirvani et al. 2017). As for phytopathogenic fungi, the most evaluated were *A. niger* (12 cases) (Barreto et al. 2016, Ben-Fadhel et al. 2017, Hemalatha et al. 2017), followed by *R. tolonifera* (Pola et al. 2016; Ramos-García et al. 2012), and *Penicillium expansum* Link. (Gniewosz et al. 2013; Guerra et al. 2016).

The studied model varied between coatings and films. On coatings, evaluations were conducted on cherry tomato (*Solanum lycopersicum* var. *cerasiforme* (Dunal) D.M. Spooner, G.J. Anderson & R.K. Jansen.) (Barreto et al. 2016), grape (*V. labrusca*) (dos Santos et al. 2012), tomato (*S. lycopersicum* L.) (Ramos-García et al. 2012), broccoli (*Brassica oleracea* var. *Itálica* Plenck.) (Ben-Fadhel et al. 2017), avocado (*Persea americana* Mill.) (Bill et al. 2014), and strawberry (*Fragaria × ananassa* (Duchesne ex Weston) Duchesne ex Rozier.) (Vu et al. 2011). On the other hand, 88.8% of the work with films was carried out in vitro and a few in carrots (*Daucus carota* L.) (Gniewosz et al. 2013).

Table 3. Biodegradable polymers added with EOs for the development of coatings and films, and their evaluation on phytopathogenic fungi.

| Polymeric matrix | EOs | Type | Phytopathogenic fungi | Assay | Reference |
|---|--|------|--|---------------|------------------------|
| Alginate (1.3 g) Glycerol (1.6%) Sodium diacetate (0.5%) Natamycin (0.008%) Sunflower oil (0.1%) Tween 80: Span 20 (0.13%) | <i>Cymbopogon citratus</i> (0.03%) | C | <i>Aspergillus niger</i> | Broccoli | Ben-Fadhel et al. 2017 |
| Chitosan (16 mg mL ⁻¹) | <i>Origanum vulgare</i> (1.25 µg mL ⁻¹) | C | <i>Rhizopus stolonifer</i> <i>Aspergillus niger</i> | Cherry tomato | Barreto et al. 2016 |

| Polymeric matrix | EOs | Type | Phytopathogenic fungi | Assay | Reference |
|---|---|------|--|------------------|--------------------------|
| Chitosan (4 y 8 mg mL ⁻¹) | <i>Mentha piperita</i> L., <i>Mentha × villosa</i> Huds. (1.25, 2.5, 5.0 µL mL ⁻¹) | C | <i>Aspergillus niger</i> <i>Botrytis cinerea</i> <i>Penicillium expansum</i> <i>Rhizopus stolonifer</i> | Grape | Guerra et al. 2016 |
| Chitosan (4 mg mL ⁻¹) | <i>Mentha piperita</i> , <i>Mentha × villosa</i> (1.25, 2.5 µL mL ⁻¹) | C | <i>Aspergillus niger</i> <i>Botrytis cinerea</i> <i>Penicillium expansum</i> <i>Rhizopus stolonifer</i> | Cherry tomato | Guerra et al. 2015 |
| Chitosan (1.0%) Arabic gum (10%) <i>Aloe vera</i> (2.0%) | <i>Thymus vulgaris</i> (1:1 y 3:1) | C | <i>Colletotrichum</i> <i>gloeosporioides</i> | Avocado | Bill et al. 2014 |
| Chitosan (20 mg mL ⁻¹) | <i>Origanum vulgare</i> (1.25, 2.5, 5.0 mL mL ⁻¹) | C | <i>Rhizopus stolonifer</i> <i>Aspergillus niger</i> | Grape | dos Santos et al. 2012 |
| Chitosan (1.0%) Beeswax (0.1%) or oleic acid (1.0%) Glycerol (0.3%) | Lime Thyme (0.1%) | C | <i>Rhizopus stolonifer</i> | Tomato | Ramos-García et al. 2012 |
| Modified chitosan (2.0%) Tween 80 (0.4%) | <i>Oreganum compactum</i> <i>Thymus vulgaris</i> <i>Mentha piperita</i> <i>Cymbopogon citratus</i> (DC.) Stapf. (0.02%) | C | <i>Botrytis cinerea</i> <i>Rhizopus stolonifer</i> | Strawberries | Vu et al. 2011 |
| Chitosan (1.0 g) | <i>Ocimum basilicum</i> (0.1, 0.3, 0.5%) | F | <i>Aspergillus niger</i> , <i>A. flavus</i> <i>Fusarium</i> sp. <i>Penicillium</i> sp. | <i>In vitro</i> | Hemalatha et al. 2017 |
| Chitosan (1 g) Tween 20 (0.5%) | Oregano + Thyme (1.0%) | F | <i>Aspergillus niger</i> | <i>In vitro</i> | Kana and Meimandi 2017 |
| Ethylene vinyl alcohol copolymer (13 g) | <i>Origanum vulgare</i> <i>Cinnamomum zeylanicum</i> (10%) | F | <i>Aspergillus flavus</i> , <i>A. parasiticus</i> | <i>In vitro</i> | Mateo et al. 2017 |
| Chitosan (0.4 g) Carboxymethylcellulose (0.8 g) Tween 80 (0.8 mL) Glycerol (0.2 mL) | Cinnamon (4.4, 8.8, 13.2%) Ginger (3.5, 7.0, 10.6%) | F | <i>Aspergillus niger</i> | <i>In vitro</i> | Noshirvani et al. 2017 |
| Cellulose acetate (8.0 g) Acetone (80 mL) Montmorillonite (0.2 g) | <i>Origanum vulgare</i> (0, 20, 40, 60%) | F | <i>Geotrichum candidum</i> <i>Rhizopus stolonifer</i> | <i>In vitro</i> | Pola et al. 2016 |
| Poly (lactic acid) (0.5 g) | Myrtle Rosemary Thyme (0.5, 1.5, 2.0, 5.0%) | F | <i>Aspergillus niger</i> | <i>In vitro</i> | Yahyaoui et al. 2016 |

| Polymeric matrix | EOs | Type | Phytopathogenic fungi | Assay | Reference |
|---|---|------|--|-----------------|---------------------------|
| Pullulan (7.5 g 100 mL ⁻¹) Glycerol (1.5 g 100 mL ⁻¹) | <i>Carum carvi</i> (0.12, 1.25, 2.5, 5.0, 8.0, 10 g 100 mL ⁻¹) | F | <i>Aspergillus niger</i> <i>Penicillium expansum</i> | Carrot | Gniewosz et al. 2013 |
| Amaranth flour (4.0%) Chitosan (1.5%) Corn starch (1.0 g) Glycerol (1.3%) Tween 20 (0.5%) | <i>Lippia berlandieri</i> Schauer. <i>Cinnamomum</i> <i>verum</i> J.Presl. <i>Cymbopogon</i> <i>citratum</i> (0, 0.25, 0.50, 0.75, 1.0, 2.0, 4.0%) | F | <i>Aspergillus niger</i> <i>Penicillium digitatum</i> | <i>In vitro</i> | Avila-Sosa et al. 2012 |
| Amaranth flour (4.0%) Chitosan (1.5%) Corn starch (1.0 g) Glycerol (1.3%) Tween 20 (0.5%) | <i>Lippia berlandieri</i> (0, 0.25, 0.50, 0.75, 1.0, 2.0, 4.0%) | F | <i>Aspergillus niger</i> <i>Penicillium</i> spp. | <i>In vitro</i> | Avila-Sosa et al. 2010 |
| Chitosan (0.5 g) Caffeic acid (221 mg) | <i>Cuminum cyminum</i> (100 a 500 ppm) | N | <i>Aspergillus flavus</i> | <i>In vitro</i> | Zhaveh et al. 2015 |

C = coating, F= film, N = nanogel.

CONCLUSIONS

EOs have great potential to be used in the control of phytopathogenic fungi. According to the cases reported, it was established that their effect not only occurs *in vitro* and through its direct application, but also when integrated into coatings and films. This offers a viable alternative for the development of novel technologies, such as the integration of EOs into bioactive packaging (nets, bags, etc.), which would allow their application in different stages of crop development, and perhaps by extending its antifungal effect during the shelf-life of horticultural commodities, the adverse effects of the use of chemical products on consumers and on the environment would be minimized.

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