Antifungal Effect of Essential Oils

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Abstract

Essential oils are employed in agriculture, medicine and food industries among others, due to their antimicrobial, antiviral, insecticidal and antifungal properties. In this chapter, we will focus on the control of fungal plant pathogens with essential oils. Fungal diseases in agricultural crops and forestry alter the physiology of plants, disrupting their normal functioning, reducing their yield and sometimes causing their death. Recent studies show antifungal effects of many essential oils against plant pathogenic fungi, which make them candidates for the development of new fungicidal agents. This chapter presents a review of the most recent advances in this area, as well as the future trends in this field.

Keywords: antifungal, plant pathogens, active compounds, essential oils, biotechnology

1. Introduction

Diseases caused by plant pathogens significantly contribute to annual loss in crop yield worldwide [1], being fungi the major pathogens with the greatest impact regarding diseases and crop production losses [2]. Application of chemical fungicides is the most prevalent and effective control method of these plant diseases, posing a serious threat to the environment and public health besides causing resistance in the pathogens [3]. Therefore, in recent years, there has been a clear tendency toward finding safer alternative methods for fungal disease control in agriculture [4]. In January 2009, The European Parliament agreed the text

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of a Regulation on Plant Protection Products (91/414/EC) [5]. Integrated Plant Management (IPM) is the effort to control plant diseases with alternative methods to chemical fungicide, eliminating or controlling their use and implementing the application of alternative control methods such as natural fungicidal substances. Therefore, the industrial research aimed at the discovery and optimization of botanical fungicides needs to address the following aspects: (a) the product must overcome resistance problems to the established commercial products, (b) the product must have lower toxicity to nontarget species and acceptable levels of persistence in the environment, and (c) the product must have market and technical advantages for the agrochemical companies [6].

Essential oils (EOs) represent a new class of crop protectants due to their effects, short shelf-life and low toxicity to the environment [7]. In addition, the probabilities of creating new resistant strains by using essential oils as fungicidal agents are low since their constituents can act as synergists [8]. Usually, mono- and sesquiterpenes such as phenols, alcohols, ethers, carbohydrates, aldehydes and ketones are the major constituents of essential oils, which are responsible for the biological activity as well as for their fragrance [9]. In fact in recent years, researchers have reported many mono- and sesquiterpene hydrocarbons as inhibitors of microbial pathogens [10]. Compounds such as carvacrol, thymol, linalool, cymene, pinene are known to exhibit antimicrobial activity [11–14]. These are the major components of essential oils with promising antifungal applications. Many essential oils have been reported as active against animal pathogenic fungi with no side effects [15–17]. Currently, there are some reviews on antifungal activity of plant extracts, generally structured according to the botanical family of plant species source of the active EOs [16] or to the active compounds of plant extracts [18]. The present review is up to date and focused on plant essential oils with antifungal activity against plant pathogenic fungi.

2. Main forest pathogenic fungi

Forest pathology deals with the diseases of forest trees, which are mainly caused by fungal and oomycete pathogens, in both their fundamental and applied aspects. The development and dissemination of effective control measures is vital to the protection of forest health. An evolution has been observed over the past few decades in terms of techniques and attitudes toward pest control. In the early 1960s, a variety of methods were used to control forest insect pests and diseases including mechanical, silvicultural, chemical and biological methods, with chemical control the most commonly used. By the 1970s, environmental concerns were being increasingly raised about the use of chemicals. As a result, research into the use of biological control agents in conjunction with silvicultural methods or pheromones began in earnest. In the last decade, integrated pest management involving a combination of control measures began to be considered the most effective way to deal with forest pests. Applications of biological control agents and microbial insecticides have become major components of pest management programs and considerable emphasis is placed on prevention and early detection as a means to avoid future pest problems [19]. There is a growing trend toward adopting more sustainable forest management strategies to contain forest pests, particularly in developed countries [20]. This movement is related to changes in the perception and role of forests, which are increasingly valued not just for economic reasons but also for their ecological and social functions. Forest insect pests, diseases and other pests are having significant impacts on forests worldwide. While the devastating impacts of indigenous forest pests are already recognized, those of introduced species are increasingly being recognized as well. Rapid transports, ease of travel, and free trade have facilitated the spread of pests [19]. A review of forest pests in both naturally regenerated forests and planted forests [19] was carried out from 2005 to 2008 in 25 countries, including a number of major forest countries (Brazil, China, Indonesia), in Africa, Asia and the Pacific, Europe, Latin America and the Caribbean, and the Near East. In this global review, the frequency of disease-causing pathogens was reported: ascomycota 59%, bacteria 3%, basidiomycota 33%, oomycota 4%, and phytoplasma 1%. In the Global Forest Resources Assessment 2010 [21], countries were also invited to list and rank up to 10 major outbreaks of insects and diseases that have occurred since 1990; the most prevalent fungal pathogens reported (in order of importance)are as follows: *Armillaria* spp., (Armillaria root disease), *Cryphonectria parasitica* (chestnut blight), *Heterobasidion* spp. (annosum root rot), *Melampsora larici-populina* (poplar rust), *Mycosphaerella pini*, (red band needle blight), *Sphaeropsis sapinea* (diplodia tip blight), *Chalara fraxinea* (ash dieback), *Gremmeniella* sp., and *Melampsora allii-populina* (poplar rust).

2.1. Emerging forest fungal diseases

In the last 15 years, two major changes affecting forest pathology—the world movement of species with trade and the rise of plantation forestry to meet growing needs of an increasing human population—have led to an increasing number of emerging diseases [21–22]. Ghelardini et al. [23] showed seven pathways driving the emergence of diseases threatening natural and planted forest ecosystems around the world: invasions by alien pathogens, climate change, emergence of new virulent and aggressive strains or species, rise of hybrid fungal species, latent and cryptic pathogens, establishment of new associations between vectors and pathogens, and the introduction of new crops and cultivation practices.

Native forests of Europe, Asia and North America have particularly suffered from invasive alien pathogens, which in the last century have caused the decline of key tree species. Among the most striking historical examples are the destruction of chestnuts by *Cryphonectria parasitica*, the alien ascomycete responsible for chestnut blight; the devastating epidemics of Dutch elm disease (DED) caused by *Ophiostoma ulmi* and *O. novo-ulmi*, two alien and highly aggressive fungi previously unknown to science; the huge damage inflicted to white pines by *Cronartium ribicola*, the invasive agent of white pine blister rust (WPBR); and the devastation of plane trees, especially obtrusive in Southern Europe, caused by the introduction of *Ceratocystis platani*, the agent of plane canker stain. In the last years, the number of described *Phytophthora* species has dramatically increased and it is now clear that forest soils host numerous and diverse resident communities of *Phytophthora* species [24]. Recently, the introduction of *Fusarium circinatum* in Spain [25] or, as a late and worrisome case, the fast-spreading epidemics of European ash dieback caused by *Hymenoscyphus fraxineus* [26–28], an anamorphic fungal pathogen with putative origin in eastern Asia [29] should be added to the list. In relation to climate change, *Phytophthora cinnamomi* is forecast to benefit from warmer winters, possibly expanding its geographic range by kilometers and reaching unaffected host populations or new host species [30]. Otherwise, [31] found that in the last 15 years, the emerging pine shoot pathogen *Diplodia sapinea* spread in France probably because of a climate shift to milder winters and wetter summers. Dutch elm disease (DED) is frequently mentioned in forest pathology reviews as the best example of a destructive disease of alien origin since it almost destroyed the elm populations of Europe, North America, and parts of Asia. DED reemerged in the 1970s in Europe as a devastating disease, which killed also elm genotypes that had been resistant in the first epidemic (at the beginning of the twentieth century). This new epidemic was caused by the emergence of the separate and highly virulent species *Ophiostoma novo-ulmi* [32] consisting of the subspecies *novo-ulmi* and *Americana* [33]. Also, [34] provided strong evidence that *Mycosphaerella populorum*, the Septoria canker of poplars, has adapted to infect, colonize, and cause mortality on poplar woody stems as a result of horizontal transfer of the necessary gene battery from ascomycete fungi associated with wood decay and from prokaryotes.

In fungal pathogens of woody plants, emergence of new interspecific hybrids was described in *Melampsora* [35], *Phytophthora* [36], *Ophiostoma* [37], *Cronartium* [38], and *Heterobasidion* [39]. An up-to-date case of a worrisome forest pathogen that may have a latency period in asymptomatic infected plants is *H. fraxineus*, the agent of European ash dieback, which penetrates into wood tissues from infected leaves and may not produce external necroses until the next growing season [40]. The *Botryosphaeriaceae* are a classical example of a very diverse group of fungi, which comprises well-studied endophytes and latent pathogens of woody plants that typically cause disease associated with some types of stress [41]. A key factor in the spread of *Diplodia sapinea* and *D. scrobiculata* is the latency period within host plant tissues. These fungi are able to live within the host without causing any visible symptoms but rapidly shift to a pathogenic interaction when an environmental stress factor primes the host (e.g., local or large-scale climate change) [31].

An example of new association between vectors and pathogens is the spread of *C. parasitica* on chestnuts by *Dryocosmus kuriphilus*, the oriental chestnut gall wasp, in Europe [42]. *D. kuriphilus* is an invasive insect of Asian origin. Also, a new association was recently reported between *D. sapinea* and *Leptoglossus occidentalis* [43], the so-called western conifer seed bug (WCSB), an invasive coreid, accidentally introduced to Italy from the US in 1999 [44], and nowadays present in several parts of Europe [45]. This association might be beneficial for both partners: the insect enables the fungus to reach a higher number and variety of host trees, either pines or other conifers, while the fungus stimulates the tree's production of monoterpenes, signaling the status of weakness of the tree and attracting more insects [43]. Regarding the new silvicultural practices, commercial plantations of poplars may be severely damaged by emerging plant pathogens worldwide [46]. In northeastern and north-central North America, one of the most harmful poplar diseases is *Mycosphaerella populorum* (Peck). Also, the epidemics of *Phytophthora ramorum* on *Larix kaempferi* (Lamb.) Carr.) in UK might have been driven by the intrinsic fragility of clonal monocultures on great areas due to ecosystem simplification, extreme mechanization, and reduced genetic diversity [47, 23]. Looking ahead, authors of [48] propose an evolutionary ecology perspective that could provide new directions for forest research or disease management: (1) fungal evolutionary diversity (species diversity of forest pathogens and their ecological niches), (2) pathogen evolution (how forest pathogens become adapted to their hosts), (3) forest resistance to disease, especially in relation to tree breeding (trade-offs, tolerance, emerging properties in populations), and (4) the role of hyperparasites and tree microbiota in the regulation of pathogen populations and disease. In this ecosystem perspective, pathogens are no longer "enemies" but are key actors of the evolution and ecology of local communities, and more generally of the ecosystem health (e.g., [49–50]).

3. Biotechnological approach: genomics-proteomics-metabolomics

Plants in nature are constantly challenged by several harmful phytopathogens including bacteria, fungi, nematodes, or virus, producing a high and negative impact on crop productivity worldwide [51]. An uncontrolled amount of synthetic and chemical pesticides used during past decades makes necessary to adopt new strategies allowing a sustainable plant protection in crops and forest systems. The use of natural compounds as plant biostimulators of growth or biotic and abiotic stress responses in plants is getting importance in the last decade because of legal restrictions on the use of phytosanitary products on crops [52, 53]. European Union policy works upon a significant reduction in pesticide use in the short future [54]. One alternative are natural origin compounds with priming capacities, such as the essential oils (EOs) [55]. This section describes examples of recent molecular approaches studying EOs and discusses the use of EOs as an alternative of nonpollutant primers to induce plant resistance for environmental-friendly plant protection.

3.1. The "priming" process

Priming is "the physiological state that enables cells to respond to very low levels of a stimulus in a more rapid and robust manner than non-primed cells. In plants, priming plays a role in defense and development" [56, 57]. A classical priming defense strategy consists in the use of very well-conserved molecules into the phytopathogen structure called damage/pathogen/microbe-associated molecular patterns (DAPMPs/PAMPs/MAMPs), such as the lipopolysaccharides (LPS, peptidoglycan (PGN), bacterial flagellin, fungal chitin, bacterial Ax21, or elongation factor Tu (EF-Tu). MAMPs are recognized by plasma-membrane receptors in plants called pattern recognition receptors (PRRs). PAMPs recognition activates a pattern-triggered immunity (PTI) associated with the increase in intracellular calcium, phosphorylation processes mediated by MAPKinase cascades, production of reactive oxygen species (ROS), plant protective compounds, induction of defense-related transcription factors, and corresponding plant pathogenesis-related proteins (PRs) such as glucanases and chitinases, as well as proteins and compounds involved in plant cell wall fortification, such as callose or lignin. PTI might be suppressed by host-adapted phytopathogens, producing an effector-triggered susceptibility (ETS), and adapted plants might block those effectors, activating a robust effector-triggered immunity (ETI) [53, 58–60]. In parallel to the PAMP response, each pathogen specifically triggers a cascade of signaling pathways mediated by phytohormone receptor and recognition of salicylic acid (SA), jasmonate acid (JA), or ethylene (ET). Commonly, it is well accepted that SA is induced by biotrophic and hemibiotrophic phytopathogens, while ET and JA are activated by necrotrophic ones and also by some hemibiotrophs. Those pathways are also interconnected, in order that the activation of one of them currently down-regulate the other one or vice versa [56]. A new mechanism called EMPIS (ETI-Mediating and PTI-inhibited sector) inhibits unnecessary immune responses in plants, limiting the fitness cost of the robust ETI, when PTI is enough effective [61]. Additionally to MAMPs, hormone-mimic-related compounds have been used as classical biostimulators of priming on plants; some examples are the synthetic chemical compounds such as: benzo (1,2,3)-thiadiazole-7carbothiolic acid (BTH), a SA analog which activates systemic acquired resistance (SAR) in crops [62], and the β-aminobutyric acid (BABA), a nonprotein amino acid priming compound with a direct fungitoxic effect [63] or the nonprotein amino acid pipecolic acid [64]. The recent advances in metabolic profiling have led to the discovering of certain new plant secondary metabolites that play significant roles as priming molecules at nature, during biotic and abiotic plant stress responses and in the plant-to-plant communication; at this point, EOs might play an important role in future biotechnological approaches [65, 66].

3.2. Metabolic engineering improving EO yield

A line of research on EO biotechnology consists in improving EO yield in plants using metabolic engineering. One of the plant species in which biotechnology approaches has been applied because its commercial interest is peppermint, and [67] transformed peppermint with various gene constructs by overexpressing genes involved in the supply of precursors through the 2C-methyl-D-erythritol 4-phosphate (MEP) pathway. The overexpression of the MEP pathway gene 1-deoxy-D-xylulose 5-phosphate reductoisomerase increased up to 78% of the oil yield over wild-type controls in a multiyear field trials. Current genetic manipulation on EO synthesis pathway was also useful improving the EO production in the same species [68]. The inhibition of the mevalonate pathway also enhanced the carvacrol biosynthesis and DXR gene expression in shoot cultures of *Satureja khuzistanica Jamzad*. *S. khuzistanica* shoots were treated with fosmidomycin (an inhibitor of the nonmevalonate pathway) and mevinolin (an inhibitor of the mevalonate pathway). The last one induced the gene expression of DXR, measured by heterologous QRT-PCR, increasing the DXR enzyme activity and allowing higher levels in carvacrol biosynthesis on plants compared to controls [69].

3.3. Molecular mechanism of EOs in fungi

Recent studies have been made in order to elucidate the molecular mechanisms underlying the phytotoxic effect for some of those compounds on phytopathogenic fungi, but still are limited. The lipophilic or hydrophobic nature of many EO components allows them to interact directly with the fungal membrane, resulting in the alteration of membrane properties including the fluidity. An active transport via trans-membrane pumps has not been yet demonstrated [55]. A recent study based on RNA-Seq-transcriptomic analysis of the fungus *Fusarium oxysporum* f. sp. *niveum*, responding to thymol, shows that most of glycosphingolipid and sphingolipid metabolism-related fungal genes were downregulated upon this treatment, while genes involved in an antioxidant activity, chitin biosynthesis, and cell wall modification were up-regulated. The authors propose that the thymol acts by disrupting fungal cell wall and cell membranes through increasing the production of ROS on the fungal cell surface as well as by blocking the fungal molecular genes necessary for cell wall fortification and cell membrane synthesis [70]. Those molecular data are in line with the results obtained by [71], showing that thymol strongly inhibited conidial production and hyphal growth on *Fusarium graminacearum* via inducing lipid peroxidation and disrupting ergosterol biosynthesis, which are essential for plasma membrane structure. A similar mechanism of action was observed on carvacrol and thymol acting against vineyard and wine spoilage yeast [72].

3.4. Plant signaling pathways and EOs

Emerging molecular studies try to elucidate the molecular effects of EOs produced by plants to the surrounding ones [52]. This old natural process is currently known as “allelopathy” or the ability of a plant to produce biomolecules, especially secondary metabolites, to affect another plant beneficially or vice versa [73]. In 1997, [74] demonstrated that methyl salicylate (MeSA), the volatile benzenoid and secondary metabolite, which is easily metabolized on the plant to SA, activates disease resistance and the expression of defense-related genes in neighboring plants and in the healthy tissues of the infected plants. Later on, other research works have shown that MeSA mediates plant-plant communications during immune responses. MeSA, which is an important insect-attracting pollinators [75], is not induced by wounding but is induced by tobacco mosaic virus and *Pseudomonas syringae* pv. *maculicola ES*4326 and *Pst DC*3000 pv. *tomato*, where both are SA inducers [76]. The plant molecular response to MeSA has been studied into essential oil extracts from Gaultheria procumbent (GEO), whose metabolic profile has been characterized recently [77]. GEO induced defense response against the hemibiotrophic fungus *Colletotrichum higginsianum* and was very effective in inducing SA plant defense-related genes similarly to the synthetic MeSA and also induced some marker genes of JA pathway [78]. A recent study investigated the role of volatile organic compounds inducing systemic acquired resistance (SAR). The headspace exposure of arabidopsis to a mixture of the bicyclic monoterpenes, α-pinene and β-pinene, induced the accumulation of ROS and the expression of SA- and SAR-related genes, including AZELAIC ACID INDUCED1 (AZI1) and three of its paralogs. Pinene-induced resistance was dependent on SA biosynthesis and signaling and on AZI1. Arabidopsis geranylgeranyl reductase1 mutants with reduced monoterpene biosynthesis were SAR-defective, but showed normal local resistance and MeSA-induced defense responses, suggesting that monoterpenes act independently of SA-mediated pathway. The volatile emissions composed by α-pinene, β-pinene, and camphene induced plant defense in neighboring plants, activating SAR responses on them. The impaired SAR immunity lines eds1-2 and *ggr*-1-1 showed reduced emissions of α-pinene, β-pinene, and camphene [79]. *Pseudomonas syringae* pv. *maculicola ES*4326 also induced terpenoid production of (E,E)-4,8,12 trimethyl-1,3,7,11-tridecatetraene (TMTT), β-ionone, and α-farnesene, depending on JA signaling and independently on SA pathway in *Medicago truncatula* [80]. Copper sulfate, which activates JA biosynthesis in plant by camalexin biosynthesis, induced VOs in arabidopsis wild-type plants but not in *tps*4 mutant showing that TMTT is induced by JA pathway [80]. TMTT and other VOs were also induced in lima beans by herbivory [81]. However, the significance on the *Pst* induction of TMTT in plants is still unknown.

4. EOs in the control of phytopathogenic fungi in agricultural crops

In agriculture, the losses caused by plant diseases reach an average of 12% per year. Among the pathogens, fungi are considered the most important. There are around 8.000 species of fungi that attack plants, distributed in more than 64 genera of fungi [82]. Added to the importance of plant diseases caused by phytopathogenic fungi, we have two other factors that must be considered.

The first concerns the constant need to produce food to feed the planet's growing population. According to the Food and Agriculture Organization of the United (FAO), global food demand in 2050 is estimated to be 60% higher than in 2006. The population living in poverty could rise from 35 to 122 million by 2030. This increase of the poor will be higher in sub-Saharan Africa, largely because of the heavy dependence of the economy of these regions on agriculture. The second factor refers to the use of pesticides. The increase in the use of pesticides is due to the increase in the cultivable area and consequently the increase in the consumption of fertilizers and pesticides. Misuse of pesticides has led to serious public and environmental health problems. The United Nations has proposed the creation of a global treaty to regulate and stop the use of pesticides in agriculture. Current patterns of production and use of pesticides are very different in each country. According to the World Health Organization (OMS), pesticides cause 200.000 deaths from poisoning each year. Almost all fatalities, or 99%, occur in developing countries. Exposure to pesticides is linked to the risk of cancer, Alzheimer's and Parkinson's disease, hormonal, developmental, and fertility problems. The rural community made up of farmers and families who live near plantations and indigenous communities is the most vulnerable. In Brazil, for example, data from the Impact of Agrochemicals on Health released in 2015 by the Brazilian Association of Collective Health (ABRASCO) show that Brazil is the largest consumer of pesticides in the world, with a 288% increase in pesticide use. The data also show that 64% of the food marketed is contaminated and that the number of poisoning by agrochemicals reaches 34.147 cases. It is believed that these statistics should be even higher due to under-reporting, i.e., subacute intoxications caused by moderate or small exposure to products of high toxicity, slow onset and subjective symptomatology, and chronic intoxications requiring months or years of exposure. Resistance of fungi to fungicides has been recorded since the 1960s. The first case of resistance was found with the use of Benomyl to control the mildew of cucurbits, caused by the fungus *Sphaerotheca fuliginea* [83] and later to control the fungus *Botrytis cinerea* in the culture of the cyclamen [84]. Since then, more than half of the known fungus species have shown some resistance to fungicides in more than 100 plant-pathogenic combinations [85]. Over the past 55 years, it has been proposed to develop agriculture under Integrated Pest Management (IPM), and this has become the main global holistic strategy for phytosanitary protection. It provides for the production of food in a sustainable agroecosystem, with the management of the soils, from the point of view of the increase in organic matter, fertility and vegetation cover, the adequate use of water for irrigation, the use of resistant varieties for different soil and climatic conditions and the use of temporal and spatial distribution of crops, the encouragement of the application of agroecology to grow food, as well as the encouragement of family agriculture, the production and preservation of creole seeds, the diversity of plant species, and reduction of pesticide use for pest and disease control as opposed to increased use of biological control. The search for biopesticides has aroused much interest from the scientific community due to the expansion of organic farming, more restrictive regulations to chemical pesticides, and the demand for healthier and safer products. Essential oils (EOs), included within the group of biopesticides of botanical origin, are complex mixtures of volatiles, mainly products of plant secondary metabolism, which comprise terpenes (mainly mono-, sesqui-, and some diterpenes) and phenolic compounds phenylpropanoids), although other groups of compounds may also occur in relevant amounts. These volatiles have aromatic components that give odor, flavor or aroma, distinct from each plant, and are part of defense mechanisms of the plant to the attack of microorganisms. Most plant species have 1–2% EOs, but in some plant species, this value can reach 10%, as in *Ocimum basilicum* [86].

4.1. Phytopathogenic fungi of agronomic interest

The antifungal properties of EOs and their constituents have been reported in several studies, most of which are due to inhibition of fungal mycelial growth in vitro. The mycelium supports all fungal activity, from the spore germination to the formation of the fruiting body, and thus represents a good indicator of fungus survival. Studies with plants of the Lamiaceae family showed positive results in the control of several phytopathogenic fungi. The EOs of oregano (*Oreganum vulgare*) and thyme (*Thymus vulgaris*) were effective against *Aspergillus niger, A. flavus, A. ochraceus, F.oxysporum, F. solani, Penicillium* sp., *Phytophthora infestans, Sclerotinia sclerotiorum, Rhizoctonia solani, B. cinerea, Monilinia fructicola, Rhizopus stolonifer, Sclerotium rolfsii, Macrophomina phaseolina*, and *Pythium* sp. [87]. *R. solani*, for example, represents an important phytopathogen of agricultural crops around the world. The fungus has a host range of more than 500 species of plants, with a complex ecology and is difficult to control. Seema and Devaki [88] studied the antifungal activity of several EOs against *R. solani* and revealed that cinnamon's EO (*Cinnamomum zeylanicum Breyne*) completely inhibited the growth of the fungus at a concentration of 500 ppm. The EOs of *T. vulgaris* [89], *Salvia fruticosa* [90], *Mentha piperita* [91–94], *Monarda* spp. [95], *Calocedrus macrolepis* var. *formosana* [96], *Bunium persicum* [94] were also effective in mycelial inhibition of the fungus. In [97], it was reported that the foliar application of *Desmos chinensis* reduced the intensity of the disease caused by *R. solani* in rice. Arici and Şanlı [98] studied the EO efficiency of *Cuminum cyminum, Anethum graveolens, Salvia officinalis, Origanum onites, Rosmarinus officinalis*, and *Lavandula intermedia* against *R. solani* and *Streptomycetes scabies* on potato and found that EO of *S. officinalis* reduced *R. solani* infection in 4.2%, and oregano's EO reduced the disease severity caused by *S. scabies* to 1.8%. Fusarium species are also important phytopathogens. The EO of *Artemisia absinthium* showed effectiveness against *Fusarium moniliforme, F. oxysporum, F. solani* [99]. Other positive results have also been reported in field experiments. Citral, methyl anthranilate, and nerol tested at the concentration of 5.0 ml/L reduced 78.1 and 80% of Cercospora (*Cercospora beticola*) and Alternaria (*Alternaria tenuis)* in sugar beet, respectively [100]. El-Mohamedy and Abd-El-latif [101] tested the EO of *T. vulgaris* applied alone or in combination with humic acid and observed a 92.2% reduction in tomato blight caused by *P. infestans* when tested at the concentration of 6.0 ml/L. In postharvest, treatment with EOs of basil (*Ocimum basilicum* L.), fennel (*Foeniculum sativum* Mill.), lavender (*Lavandula officinalis* Chaix), marjoram (*O. majorana* L.), oregano (*O. vulgare* L.), mint (*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.) was effective against *B. cinerea* and *Penicillium expansum* [102]. Al-Reza et al. [103] tested the EO of *Cestrum nocturnum* L. at 1000 ppm concentration and showed that EO inhibited up to 80.6% growth of *B. cinerea, Colletotrichum capsici, F. oxysporum, F. solani, P. capsici, R. solani*, and *S. sclerotiorum*. The EO of *C. nocturnum* also inhibited the spore germination and reduced the disease by 82–100% in pepper seedlings. Muchembled et al. [104] studied some OEs against *Venturia inaequalis* strains of apples with different sensitivities to Tebuconazole compared to the application of copper sulfate and highlighted the effectiveness of clove EOs

(*Syzygium aromaticum*), eucalyptus (*Eucalyptus citriodora*), mint (*Mentha spicata*), and savory (*Satureja montana*) with priority components such as eugenol and carvacrol. They also found that each strain of the fungus reacted differently to each treatment, indicating that each strain of the pathogen had different survival mechanisms.

5. Industrial applications in agronomy and agrifood industry

Essential oils are employed in agriculture, medicine, and food industries among others, due to their antimicrobial, antiviral, insecticidal, and antifungal properties. They are specially employed in agriculture, against phytopathogenic fungi such as *Aspergillus, Penicillium, Fusarium, Rhyzoctonia,* and other fungi, which produce many loses in agronomic crops. Also, these fungi are pathogenic to many forest species, and nowadays, we are losing many trees due to these fungi. Essential oils and their effect as antifungal agents must be approached from a biotechnological point, taking in account their genomic, proteomic, and metabolomic functioning. Finally, industrial and commercial applications are being developed, so these products can reach easily their target and have the desired effect for which they are designed. Antimicrobial volatile substances from plants, such as essential oils (EOs) present an alternative to chemical fungicides and food preservatives. Their main new uses and industrial applications of essential oils as antifungals in agronomic crops and in the agrifood industry are the pre- and postharvest treatment of vegetables such as fruits and grains in order to prevent their decay and increase their time of storage, to protect seeds against fungal attack, to prevent food spoilage due to fungal attack, and to produce active containers for vegetables and other food.

Eos are employed to avoid pre- and postharvest fungal diseases of vegetables, but their stability, solubility, and bioavailability are limited and the use of EOs as antifungal agents is limited due to the degrading ability of these volatile compounds under the action of heat, pressure, light, and oxygen. In addition, the fact that there are not water soluble limits their use in certain applications, especially when a controlled release is required [105]. Also, it must be considered that the application of these natural products may alter the characteristics of food, such as aroma or taste, so this is another factor which to be taken in account. The main ways of application of EOs as fungicidal in the agrifood industry, from crops to preservation, are emulsions, encapsulation, and vapor application. All these biotechnologies allow a good contact of the EOs with the plant, a time controlled release, and avoid the alteration of the properties of vegetables. It must be also taken in account that the antifungal effect of the EOs depends on the application method. Suhr and Nielsen [106] have studied how larger phenolic compounds such as thymol and eugenol (from thyme, cinnamon and clove) have best effect against rye bread spoilage when applied directly to the medium, whereas other smaller compounds such as allyl isothiocyanate and citral (from mustard and lemongrass) are most efficient when added as volatiles.

EOs can be prepared into **emulsions** by different techniques. **Microemulsion** of EOs is prepared with EO, Tween 20, and ethanol, and can be unlimitedly diluted with water, being stable for long time. *Laurus nobilis* EO has been proven to be effective in cherry tomatoes applied in this way [107]. **Nanoemulsions** of thymol without carrier oil have also been studied to avoid the deployment of wheat due to *Fusarium gramineum* [108]. **Double w/o/w emulsion** type prepared lipophilic and hydrophilic emulsifiers with xanthan gum as thickener showed stability and water-dilution tolerance and retained most of the electrolytes included in the internal aqueous phase. Antifungal activity of the EOs increased, and the absence of organic solvents makes these formulations environmentally safe. Also, the property of controlled electrolyte release makes these formulations attractive [109].

The **microencapsulation** in porous materials allows direct contact between the fungus and the microparticle in the soil, which acts more efficiently against the fungus. That is, these could be put directly into the crop acting as biopesticides throughout the growth of the vegetables. Microencapsulation can be done by simple coacervation [110–111] and it has been tried already in fruits such as mango with thyme and rosemary EOs [110] and to preserve peanut seeds with *Lippia turbinata* EO [111]. Carvacrol and thymol from oregano and thyme have also been studied in microcapsules of mesoporous silica and B-cyclodextrin, together with cinnamaldehyde and eugenol from cinnamon and clove, respectively. **Nanoencapsulation** is also used to enhance antifungal activity and stability of the oils against fungi. Nanoencapsulation in chitosan nanoparticles (CSNPs) is done by an ionic gelation technique. This technique has shown a controlled and sustained release of EOs for 40 days in comparison with unmodified EOs [101]. Nanoparticle carriers of EOs, as compared to microsize carriers, show a better surface area rate, solubility, bioavailability, controlled release, and targeting of the ingredients [101]. Nanoencapsulation of EOs has been studied also for their incorporation into fruit juices to prevent fungal activity while not affecting on the quality attributes of the product [112].

Simple vapor application of EOs can change the sensory profile of fruits and vegetable [113–114]. EOs from cinnamon (*Cinnamomum zeylanicum* Nees.), thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.), clove (*Syzygium aromaticum* L.), lemongrass (*Cymbopogon citratus* [DC] Stapf.), and ginger (*Zingiber officinale* Rosc.) have shown to inhibit the growth of Aspergillus spp. in oats [114]. But furthermore, there are new technologies of application of the EOs, such as the combination with **warm air flow (WAF)**, that can be used in the control of postharvest fungal pathogens of grains [115], being more effective compared to standard vapors in disc volatilization [113] with very low effect on their sensory profile.

EOs are a very good source of natural additives for **active packaging (films & coatings)**, which refers to the incorporation of additives into the packaging material, maintaining its properties without adding active agents in the food product, thus reducing the use of aggressive techniques and synthetic chemicals in food. Oregano is one of the EOs that has been positively tested in this way [116]. In that sense, chitosan **composite films** enriched with essential oils of cinnamon, thyme, clove, and lime alone or in combination have been tried against *Colletotrichum gloeosporioides* in papaya fruit. This coating can be an alternative to potentially reduce the need for cold storage during postharvest handling [117]. Edible coatings with oregano EO have been proved for the preservation of tomatoes against *Alternaria alternata* growth maintaining the sensorial acceptability of tomatoes [118].

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