

Review

Antifungal Mechanisms of Plant Essential Oils: A Comprehensive Literature Review for Biofungicide Development

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Abstract

Plant pathogenic fungi pose a persistent global threat to food security, causing severe yield losses in staple crops and increasing dependence on chemical fungicides. However, the ecological and toxicological drawbacks of synthetic fungicides have intensified the search for safer, plant-derived alternatives. This review synthesizes current advances on the antifungal mechanisms of plant essential oils (EOs) and their prospects for biofungicide development. The literature reveals that the antifungal activity of EOs arises from their diverse phytochemical composition, principally terpenes, phenolics, and aldehydes that target multiple fungal cellular sites. These compounds disrupt membrane integrity through ergosterol depletion, inhibit chitin and β -glucan synthesis, interfere with mitochondrial energy metabolism, and induce oxidative stress, leading to lipid peroxidation and cell death. Morphological and transcriptomic evidence confirms that EOs alter hyphal growth, spore germination, and key gene expression pathways associated with fungal virulence. Furthermore, emerging nanotechnological and encapsulation strategies enhance EO stability, bioavailability, and field persistence, addressing major barriers to their large-scale agricultural application. The integration of EO-based biofungicides within sustainable and precision agriculture frameworks offers a promising route to reduce chemical inputs, mitigate resistance development, and promote ecological balance. This review underscores the need for interdisciplinary research linking phytochemistry, nanotechnology, and agronomy to translate EO-based antifungal mechanisms into next-generation, environmentally compatible crop protection systems.

Keywords: antifungal; activity; sustainable agriculture; membrane integrity; oxidative stress



Academic Editors: Inmaculada Izquierdo-Bueno and Javier Moraga

Received: 30 September 2025

Revised: 22 October 2025

Accepted: 29 October 2025

Published: 5 November 2025

Citation: Leiva-Mora, M.; Bustillos, D.; Arteaga, C.; Hidalgo, K.; Guevara-Freire, D.; López-Hernández, O.; Saa, L.R.; Padilla, P.S.; Bustillos, A.

Antifungal Mechanisms of Plant Essential Oils: A Comprehensive Literature Review for Biofungicide Development. *Agriculture* **2025**, *15*, 2303. <https://doi.org/10.3390/agriculture15212303>

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1. Introduction

Plant pathogenic fungi continue to represent one of the most persistent threats to global agriculture, causing substantial yield losses in crops of high economic and nutritional relevance, including wheat, rice, maize, and potatoes [1]. To mitigate these impacts, farmers have historically depended on the use of chemical fungicides and other synthetic control agents as part of intensive disease management programs [2]. Although these compounds have proven highly effective in reducing the prevalence of fungal infections, their prolonged and excessive use has generated new challenges that extend far beyond plant protection.

The intensive application of synthetic fungicides has become a paradigm of growing concern in modern agriculture. Their widespread use has not only altered the ecological balance of agroecosystems but has also introduced significant human health and socio-economic risks. Residual chemical compounds persist in food commodities, posing latent dangers to consumers, while their non-target effects compromise beneficial soil microorganisms, aquatic ecosystems, and pollinators essential to ecological stability [3]. In addition, the increasing financial burden associated with purchasing these inputs, together with the emergence of resistant fungal strains, creates a vicious cycle that weakens the economic sustainability of agricultural systems [4,5].

Within this scenario, the limitations of conventional fungicides have become increasingly evident. Continuous exposure to chemical molecules has driven the evolution of resistant fungal populations, diminishing the effectiveness of widely used fungicides such as triazoles, strobilurins, and dithiocarbamates [6]. Moreover, their environmental persistence, bioaccumulation, and toxicity to non-target organisms have raised serious regulatory and ethical concerns. As agriculture undergoes a global transition toward sustainability, these limitations underscore the necessity of developing innovative, eco-friendly alternatives that maintain efficacy while reducing environmental impact [7].

In response to these challenges, plant-derived essential oils (EOs) have emerged as promising candidates for the development of next-generation biofungicides. These natural, volatile compounds are synthesized by aromatic plants as part of their secondary metabolism and are characterized by a complex mixture of terpenes, phenolics, aldehydes, and alcohols [8].

Their broad-spectrum biological activity, biodegradability, and low environmental persistence make them attractive for integration into sustainable crop protection systems. Numerous studies have demonstrated that EOs exert potent antifungal effects by disrupting the fungal cell membrane, inhibiting enzyme activity, and inducing oxidative stress that culminates in cell death [9]. For instance, thyme (*Thymus vulgaris*) essential oil, rich in thymol and carvacrol, has exhibited strong inhibitory activity against *Botrytis cinerea* and *Fusarium oxysporum*, two of the most economically damaging plant pathogens [10,11].

Recent research has expanded our understanding of the mechanistic complexity of these natural products, revealing that their antifungal action involves not only direct toxicity but also modulation of gene expression and interference with fungal development pathways [12]. Furthermore, advances in nanotechnology and formulation science are enhancing EO stability, controlled release, and bioavailability, paving the way for their practical application under field conditions.

Given this context, the present review aims to critically analyze and synthesize recent research concerning the antifungal mechanisms of plant essential oils and their potential role in developing innovative, sustainable biofungicides. By integrating biochemical, morphological, and molecular evidence, this work seeks to provide a comprehensive framework that supports the rational design of EO-based products capable of replacing or complementing synthetic fungicides in modern agriculture. Ultimately, this review underscores that the strategic combination of plant-derived antifungal compounds with

advanced formulation and delivery systems represents a crucial step toward achieving environmentally responsible and resilient crop protection.

2. Essential Oils

Essential oils (EOs) are concentrated, volatile compounds extracted from various parts of aromatic plants, including leaves, flowers, stems, and roots [13]. They have complex chemical composition with a diverse array of phytochemicals such as terpenes, phenolics, and aldehydes, contributing to their distinctive aromas and flavors [14].

EOs are not only valued for their aromatic properties but also for their rich chemical compositions, which include a great variety of phytochemicals such as terpenes, phenolics, and aldehydes [15]. This diverse array of compounds not only defines their unique scents and flavors but also underpins their biological activities, including antifungal properties [16].

The antifungal activity of EOs has garnered considerable attention in recent years, as researchers explore their potential as biofungicides [17]. Studies have demonstrated that they effectively inhibit the growth of various plant pathogenic fungi, including species from genera such as *Botrytis* [18].

Eos, besides their antifungal properties, offer the advantage of a lower environmental impact compared to conventional chemical fungicides [19]. Their natural origins and biodegradability position them as promising alternatives in integrated pest management (IPM) strategies [20].

Great potential is expected for EOs when they are used in combination with other biological control agents, which could enhance their effectiveness and broaden their application spectrum [21]. Such synergistic approaches not only improve disease management but also contribute to the overall sustainability of agricultural practices, aligning with the increasing demand for environmentally friendly solutions [22].

3. Antifungal Properties of Plant Essential Oils Against Fungal Pathogens

The antifungal properties of plant essential oils have emerged as a significant area of research, particularly in their capacity to combat both human and plant pathogenic fungi [23]. Essential oils derived from a diverse range of plants, such as tea tree, oregano, and *Eucalyptus*, have demonstrated substantial antifungal activity against various fungal species [24].

The essential oil of *Melaleuca alternifolia* (tea tree) is well documented for its efficacy against dermatophytes and other opportunistic pathogens in humans, including *Candida albicans* [25]. The bioactive compounds within these oils, such as terpinen-4-ol and α -terpineol, play critical roles in disrupting fungal cell membranes and inhibiting key metabolic pathways, thereby leading to fungal cell death [26].

In the context of agriculture, EOs are gaining recognition for their effectiveness against economically significant plant pathogens [27]. Oils such as thyme and clove have shown remarkable antifungal activity against fungi like *Botrytis cinerea* and *Fusarium oxysporum* which are responsible for substantial crop losses [28].

EOs derived from oregano, thyme, and clove have demonstrated antifungal effects on various *Fusarium* species, including *Fusarium oxysporum* and *Fusarium solani* [29]. These oils contain bioactive compounds, particularly phenolic compounds and terpenes can lead to reduced fungal biomass and spore germination, highlighting their potential as effective biofungicides in agricultural settings [30].

EOs derived from plants such as oregano and thyme have demonstrated significant inhibitory effects on the growth and spore germination of *Alternaria linariae*, the causal agent

of early blight in tomato plants [31]. Thyme essential oil (TEO) enhances the activity of defense related enzymes peroxidase, polyphenol oxidase and β -1,3-glucanase and promote phenolic compound accumulation indicates a dual mode of action both direct antifungal activity and the induction of systemic resistance in tomato plants. TEO was considered a promising product with multiple effects and high potential for biological control of early blight in tomato plants [32].

The antifungal activity of oregano essential oil against *Rhizoctonia solani* has been well documented, establishing it as an effective natural biofungicide. With an effective concentration (EC50) of 22.89 $\mu\text{g/mL}$, oregano essential oil not only inhibits mycelial growth but also induces significant alterations in mycelial morphology. Ultrastructural analyses reveal that the oil impacts the fungal cell membrane, leading to increased permeability and integrity loss [33].

Some essential oils (EOs) had antifungal activity against *Colletotrichum lindemuthianum* the causal agent of the common bean anthracnose [34]. Induced defense enzymes like Chitinase (CHI), Polyphenol oxidase (PPO), Guaiacol peroxidase (POX) and Phenylalanine ammonia-lyase (PAL) were activated on bean plants after essential oil were applied [35]. Additionally, EOs damaged the conidia's ultrastructure by causing vacuolization, cytoplasmic leakage, and plasma membrane invagination of *C. lindemuthianum* isolates [34].

The in vitro antifungal activity of nine monoterpenes against *Sclerotinia sclerotiorum* has revealed that carvacrol is a particularly potent antifungal agent, demonstrating strong toxicity. When *S. sclerotiorum* was treated with carvacrol, the mycelial surface exhibited severe morphological alterations, characterized by a shriveled, uneven texture and the leakage of cytoplasmic contents, indicative of cellular damages [36].

Evaluation of the efficacy of essential oils (EOs) from grapefruit, rosemary, pine, sage, and thyme against *Cercospora beticola* indicated that thyme EO exhibited the highest antifungal activity, with a minimum inhibitory concentration (MIC) of 0.313 mL/L for most isolates tested. Moreover, thyme EO effectively inhibited the growth of multi-resistant *C. beticola* strains against chemical fungicides, highlighting its potential as a biocontrol agent [37].

4. Antifungal Mechanisms of Biofungicides Based on Essential Oils

4.1. Fungal Cell Membrane Disruption and Structural Damage

Essential oils (EOs) exhibit diverse chemical compositions that confer a wide range of biological activities, including potent antifungal properties [38]. Among their principal mechanisms of action, disruption of the fungal cell membrane is the most extensively documented. Terpenoids, phenolics, and aldehydes such as thymol, carvacrol, and citral interact with the lipid bilayer of fungal membranes, altering their fluidity and permeability [39]. This alteration promotes leakage of intracellular constituents, ionic imbalance, and ultimately the collapse of membrane integrity [26].

The hydrophobic compounds present in EOs embed within the phospholipid bilayer, displacing essential sterols such as ergosterol and key phospholipids, thereby weakening the overall structural framework of the fungal cell envelope [40]. Recent findings demonstrate that thymol and carvacrol specifically target ergosterol, a pivotal sterol for fungal membrane stability, which accounts for their selective antifungal activity [41].

Figure 1 schematically illustrates the antifungal mechanisms of plant-derived essential oils, highlighting their interactions with fungal cell walls, membranes, and intracellular organelles. The diagram emphasizes how EOs trigger cellular leakage, oxidative imbalance, and enzymatic inhibition that collectively lead to fungal cell death.

Furthermore, EOs often act synergistically with conventional fungicides, potentiating antifungal efficacy while mitigating resistance development [42]. This synergy enables

lower dosages and reduces environmental residues, aligning EO-based strategies with the principles of sustainable agriculture [43].

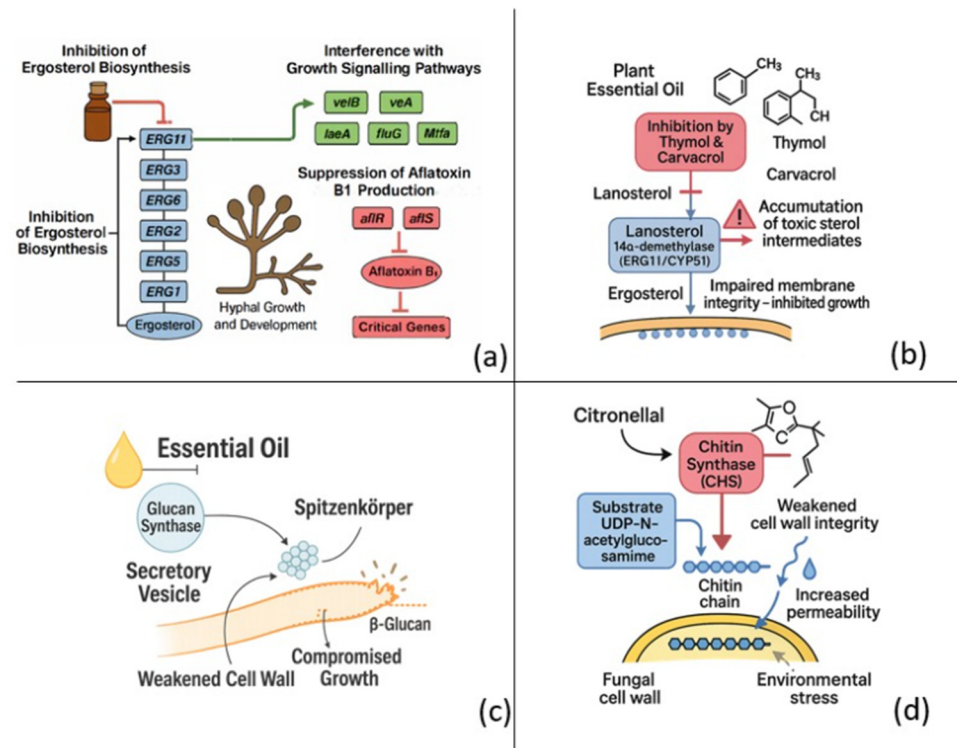


Figure 1. Antifungal targets of plant-derived essential oils (EOs). (a) Multi-target blockade of ergosterol biosynthesis (ERG11, ERG3, ERG6, ERG2, ERG5, ERG1) coupled to interference with the *velB/vea/laeA/fluG/MtfA* signaling module and down-regulation of the *aflR/aflS* activators, collectively inhibiting hyphal growth and aflatoxin B₁ production. (b) Phenolic monoterpenes thymol and carvacrol inhibit lanosterol 14α-demethylase (ERG11/CYP51), causing lanosterol accumulation, toxic sterol build-up and loss of membrane integrity. (c) EO exposure prevents delivery of glucan synthase-containing secretory vesicles addressed to the fungal membrane, depleting β-glucan at the hyphal tip and weakening the cell wall. (d) The aldehyde citronellal binds chitin synthase (CHS), blocks incorporation of UDP-N-acetyl-glucosamine into nascent chitin chains and increases wall permeability, ultimately promoting osmotic stress and lysis. Together, these mechanisms highlight the antifungal potential of EOs to compromise fungal viability by simultaneously disrupting membrane sterol homeostasis, cell wall polysaccharide synthesis.

To illustrate the breadth of antifungal action, Table 1 summarizes representative studies reporting the inhibitory effects of EOs against key plant pathogens. The table highlights the diversity of EO sources, targeted fungal species, and observed disease suppression outcomes under both laboratory and field conditions. These interactions collectively underscore the potential of EOs as eco-friendly alternatives to synthetic fungicides.

Table 1. Antifungal activity of plant-derived essential oils against major phytopathogenic fungi and associated crop diseases.

Essential Oil Source	Target Plant Pathogenic Fungi	Diseases	Control	Reference
(<i>Origanum vulgare</i>)	<i>Botrytis cinerea</i>	Pepper fruit rot	Lesion growth (cm ²) reduced	[44]
Thyme (<i>Thymus vulgaris</i>)	<i>Alternaria alternata</i>	Softening of fruits (especially grapes and citrus)	Inhibition of growth	[45]

Table 1. Cont.

Essential Oil Source	Target Plant Pathogenic Fungi	Diseases	Control	Reference
Clove (<i>Syzygium aromaticum</i>)	<i>Fusarium oxysporum</i> and <i>Fusarium solani</i>	Root rot disease of cucumber seedling and seed rot.	Reduced seed rot and mortality percentage of seedling cucumber	[46]
<i>Eucalyptus globulus</i>	<i>Fusarium graminearum</i>	Wheat <i>Fusarium</i> Head Blight	Decreased <i>Fusarium</i> Head Blight infection rate	[47]
<i>Cymbopogon citratus</i>	<i>Colletotrichum lindemuthianum</i>	Common bean anthracnose	Reduced anthracnose severity	[48]
Thyme (<i>Thymus vulgaris</i>)	<i>Cercospora beticola</i>	<i>Cercospora</i> Leaf Spot of Sugar Beets (<i>Beta vulgaris</i>)	Reduced infected Leaf Area [%]	[49]
Cedarwood (<i>Cedrus atiantica</i>)	<i>Rhizoctonia solani</i>	Sheath blight of rice	Per cent of yield increased	[50]
Celery (<i>Apium graveolens</i>)	<i>Podosphaera fusca</i>	Powdery mildew of cucumber (<i>Cucumis sativus</i>)	Reduced severity of powdery mildew in cucumber seedlings	[51]
<i>Syzygium aromaticum</i>	<i>Rhizoctonia solani</i> , <i>Sclerotinia sclerotiorum</i> , <i>Fusarium oxysporum</i> , and <i>Fusarium solani</i>	Root Rot and Wilt of Marigold (<i>Calendula officinalis</i> L.)	Reduced Damping-Off %, Root Rot % and Wilt %	[52]
<i>Citrus x limon</i>	<i>Phytophthora</i> spp.	Brown Rot of Cocoa	Reduced severity of symptoms of cocoa brown rot	[53]

4.2. Enzymatic and Molecular Inhibition of Key Fungal Pathways

The antifungal efficiency of essential oils (EOs) is closely related to their capacity to inhibit key enzymatic systems involved in fungal metabolism, energy production, and cell-wall biogenesis. Table 2 summarizes the main enzymatic targets reported in the literature, detailing their substrates, regulatory pathways, and the resulting cellular effects that contribute to growth suppression and loss of viability.

Beyond physical disruption, EOs exert biochemical interference by inhibiting critical fungal enzymes involved in energy production, sterol biosynthesis, and cell wall construction. Ajwain essential oil, for example, suppresses ergosterol biosynthesis and downregulates regulatory genes in *Aspergillus flavus*, including *velB*, *veA*, *laeA*, *fluG*, and *Mtfa*, as well as developmental genes *brlA* and *abaA* [54]. It also interferes with *aflR* and *aflS*, key activators of aflatoxin biosynthesis.

Other EO components inhibit essential enzymes such as lanosterol demethylase [55], and glucan synthase [56], Chitin synthase [57]chitin synthase [58], thereby impeding the synthesis of chitin and β -glucans major structural polymers of fungal cell walls. Inhibition of these enzymes compromises rigidity, increases permeability, and renders fungi more vulnerable to environmental stress.

Mitochondrial enzymes are also major EO targets. Cytochrome c oxidase (Complex IV) inhibition disrupts electron transfer and ATP synthesis, leading to energy deprivation and the accumulation of reactive oxygen species (ROS). Likewise, NADH dehydrogenase inhibition halts oxidative phosphorylation, resulting in metabolic imbalance and oxidative stress [58].

Table 2. Primary enzymatic targets for essential oil antifungal activity: substrates, cellular effects, and modes of action.

Enzyme	Substrate	Active Compound	Regulatory Pathway	Fungal Cellular Effect	Mechanism of Kinetic Enzymatic
Chitin Synthase	UDP-N-acetylglucosamine	Chitin	Transcriptional regulators (chs1, chs2 in <i>S. Pombe</i>), Transcription factors like Rlm1	Disruption of cell wall integrity, increased permeability, and susceptibility to stress.	Interaction with the enzyme's active site, preventing substrate binding and enzymatic activity.
Lanosterol Demethylase	Lanosterol	Ergosterol (via demethylation)	Part of the ergosterol biosynthetic pathway	Accumulation of toxic sterol intermediates, impedes fungal growth.	Inhibition by compounds like thymol and carvacrol (specific kinetic mechanism not detailed).
Glucan Synthase	UDP-glucose	β -(1,3)-Glucans	Allosteric Activation, the binding of Rho1-GTP to Fks1 induces a conformational change that increases the enzyme's catalytic activity (Vmax), enabling rapid glucan polymer synthesis at specific sites, most notably the growing hyphal tip.	Loss of cell wall rigidity, cellular lysis, and death due to osmotic stress.	Disruption of enzyme activity, compromising the synthesis of vital cell wall components.
Cytochrome c Oxidase	Cytochrome c, O ₂	H ₂ O	Final step of the mitochondrial electron transport chain.	Reduced ATP production, accumulation of ROS, oxidative stress.	Inhibition disrupts electron transfer to oxygen, reducing the proton gradient for ATP synthesis.
NADH Dehydrogenase	NADH, Ubiquinone	NAD ⁺ , Ubiquinol (reduced)	Initiates the mitochondrial electron transport chain	Diminished ATP production, metabolic dysregulation, increased oxidative stress.	Binding of EO components blocks substrate access and prevents electron transfer.
Catalase (CAT)	H ₂ O ₂	H ₂ O, O ₂	Key component of the antioxidant defense system.	Accumulation of H ₂ O ₂ , leading to oxidative damage and cell death	Enzyme inhibition, impairing the cell's ability to degrade hydrogen peroxide.
Superoxide Dismutase (SOD)	Superoxide (O ₂ ⁻)	H ₂ O ₂ , O ₂	Key component of the antioxidant defense system.	Accumulation of superoxide radicals, leading to oxidative damage and cell death.	Enzyme inhibition, impairing the cell's ability to neutralize superoxide radicals.
Phospholipases	Phospholipids	Fatty acids	Involved in membrane integrity, lipid metabolism, and signaling.	Reduced virulence, inhibition of growth, compromised membrane function.	Inhibition of enzymatic activity by compounds like terpinen-4-ol and eugenol.

Additionally, EOs inhibit antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD), enhancing intracellular ROS accumulation and oxidative injury [59]. Compounds like terpinen-4-ol and eugenol also block phospholipases, compromising lipid metabolism and cellular signaling [60].

Several terpenes directly target the mevalonate pathway, the metabolic route responsible for ergosterol synthesis, by inhibiting HMG-CoA reductase (HMGR) [61], mevalonate-5-pyrophosphate decarboxylase (MVD) [62], and farnesyl pyrophosphate synthase (FPPS) [63]. Moreover, lanosterol synthase (LSS) [64], C-14 sterol demethylase (CYP51) [65], and C-24 methyltransferase (ERG6) [66] are key enzymatic targets through which EO constituents especially thymol and carvacrol impair ergosterol biosynthesis and membrane assembly.

As summarized in Table 3, the antifungal efficiency of EOs is closely linked to the structural diversity of their secondary metabolites. The table outlines the principal biosynthetic

pathways namely the mevalonate (MEV) and methylerythritol phosphate (MEP) routes, alongside representative metabolites and their proposed biochemical targets. Understanding these pathways clarifies how EOs interfere with vital fungal metabolic systems.

Table 3. Major biosynthetic pathways and metabolites responsible for antifungal activity in plant essential oils.

Metabolite Class	Biosynthetic Pathway	Key Metabolite	Plant Source	Proposed Antifungal Mechanism of Action
Phenolic Monoterpenes	MEP Pathway Cyclization/Aromatization	Timol, Carvacrol	(<i>Origanum vulgare</i>), Tomillo (<i>Thymus vulgaris</i>)	Membrane destabilization, increased permeability, ion and cell content leakage, inhibition of ergosterol biosynthesis.
Phenylpropanoids	Shikimate-Phenylpropanoid Pathway	Eugenol, Chavicol, Cinnamaldehyde	Clove (<i>Syzygium aromaticum</i>), Basil, Cinnamon (<i>Cinnamomum zeylanicum</i>)	Membrane depolarization, lipid peroxidation, inactivation of essential enzymes, induction of oxidative stress.
Oxygenated Monoterpenes	MEP Pathway Oxidation (P450)	Linalool, Citral, 1,8-Cineole (Eucalyptol), Geraniol, Menthol, Pulegone	Basil, Lemongrass, Lavender, <i>Eucalyptus</i> , Palmarosa, Mint	Alteration of membrane fluidity, interaction with membrane proteins, inhibition of spore germination, ion channel disruption.
Sesquiterpenes	MEV/MEP Pathway	Farnesol, β -Caryophyllene, Nerolidol, α -Bisabolol	Ginger, <i>Eucalyptus</i> , Jasmine, Cabreuva, Chamomile	Inhibition of yeast-to-hypha transition, alteration of fungal morphogenesis, synergy with other compounds, biofilm inhibition.
Aliphatic Aldehydes	Fatty Acid Pathway	Octanal, Decanal, Nonanal	Orange, Lemon, other Citrus peels	Membrane disruption, non-specific enzyme inhibition, induction of metabolic paralysis in fungal cells.
Monoterpene Hydrocarbons	MEP Pathway	(+)-Limonene, α -Pinene, γ -Terpinene, p-Cymene	Citrus peels, Pine, Turpentine, Cumin	Solubilization in membrane lipids, causing swelling and leakage; often act synergistically with oxygenated terpenes.
Allyl Isothiocyanates	Glucosinolate Pathway (from amino acids)	Allyl Isothiocyanate	Mustard, Horseradish, Wasabi	Powerful electrophile that reacts with thiol groups in enzymes and proteins, disrupting cellular functions.
Aliphatic Alcohols	Fatty Acid Pathway	1-Octanol, Terpinen-4-ol	Citrus, Tea Tree (<i>Melaleuca alternifolia</i>)	Membrane disruption, inhibition of mycelial growth and spore formation, energy metabolism interference.

Figure 2 complements these data by illustrating the parallel enzymatic targets and the convergence of multiple inhibition routes that disrupt ergosterol biosynthesis and mitochondrial energy metabolism.

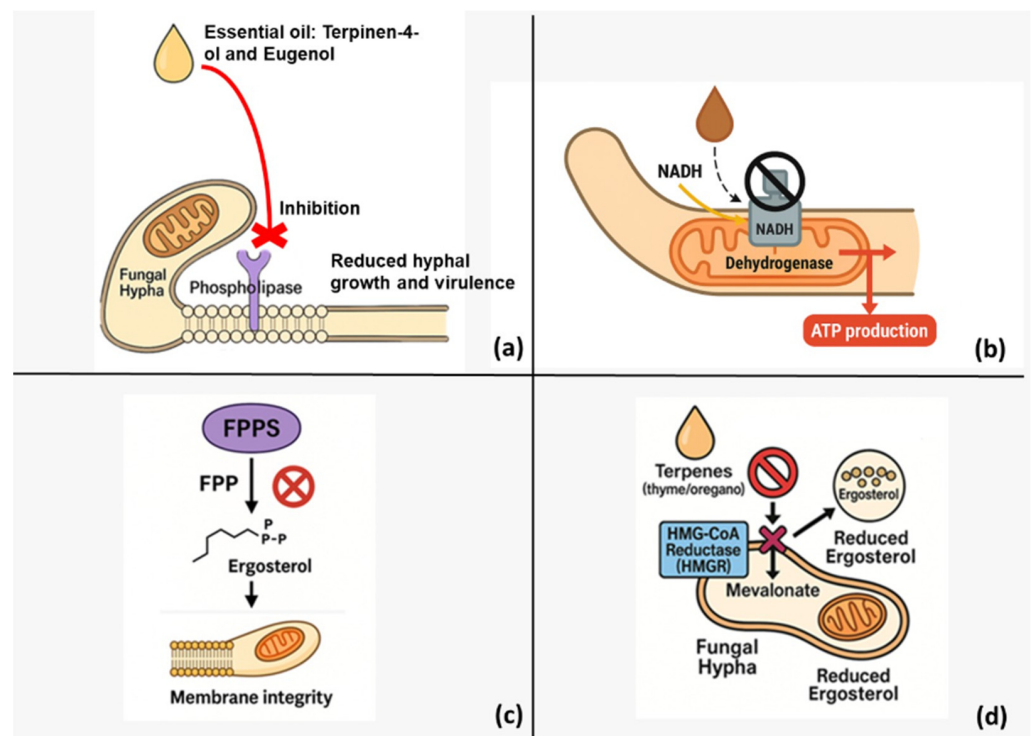


Figure 2. Multifaceted antifungal mechanisms of plant-derived essential oils (EOs). (a) Monoterpenoids terpinen-4-ol and eugenol bind to membrane-embedded fungal phospholipases, blocking phospholipid turnover and thereby weakening the cell wall, arresting hyphal extension and attenuating virulence. (b) EO constituents inhibit mitochondrial NADH dehydrogenase (Complex I), interrupting electron flow to ubiquinone, collapsing the proton-motive force and lowering ATP output. (c) Targeting farnesyl pyrophosphate synthase (FPPS) reduces the cellular pool of farnesyl pyrophosphate, limiting downstream sterol (ergosterol) synthesis and compromising plasma-membrane integrity. (d) Terpene-rich EOs from thyme and oregano inhibit HMG-CoA reductase (HMGR)—the rate-limiting enzyme of the mevalonate pathway—decreasing mevalonate flux and further depleting ergosterol. Collectively, these parallel disruptions of lipid metabolism and respiratory energy production undermine fungal growth and viability.

4.3. Morphological and Cytoskeletal Alterations in Fungi

In addition to altering fungal morphology, essential oils have demonstrated a potent capacity to inhibit spore and conidial germination across diverse phytopathogens. This effect is highly dependent on EO composition, concentration, and fungal species. Table 4 compiles recent evidence on the inhibitory potential of different essential oils against agriculturally important fungi, highlighting both complete and partial suppression under controlled experimental conditions.

Morphogenesis, the differentiation of hyphae, spores, and reproductive structures is crucial for fungal pathogenicity [74]. Essential oils disturb these morphological processes by targeting cytoskeletal components and interfering with microtubule and actin organization [75].

Clove oil, rich in eugenol, alters microtubule assembly and stability, disrupting vesicle transport and cell division, ultimately reducing fungal proliferation [76]. Actin filaments concentrated at hyphal tip are also disrupted by thymol, which affects polymerization dynamics and hinders vesicle transport and hyphal elongation [77]. These structural disturbances impair nutrient absorption, colonization capacity, and virulence.

Table 4. Effect of essential oils in spore germination of fungal plant pathogens.

Plant Fungal Pathogen	Plant Disease	Essential Oil Used	Doses	Spore/Conidial Inhibition	References
<i>Botrytis cinerea</i>	Gray Mold	<i>Origanum vulgare</i> (Oregano)	125 $\mu\text{L.L}^{-1}$	Complete inhibition	[67]
<i>Penicillium digitatum</i>	Green Mold	Cinnamon bark essential oil	106 $\mu\text{L.L}^{-1}$	Complete inhibition	[68]
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Fusarium Wilt	<i>Thymus vulgaris</i> (Thyme)	0.83 to 6.66 $\mu\text{L.mL}^{-1}$	Partial inhibition	[69]
<i>Alternaria alternata</i>	Black Spot	<i>Syzygium aromaticum</i> (Clove)	5 g. mL^{-1}	Complete inhibition	[70]
<i>Colletotrichum gloeosporioides</i>	Anthrachnose	<i>Cymbopogon citratus</i> (Lemongrass)	0.1 g. mL^{-1}	95% inhibition of conidial germination	[71]
<i>Aspergillus flavus</i>	Maize pod Rot, (Aflatoxin producer)	<i>Mentha piperita</i> (Peppermint)	0.343 $\mu\text{L.mL}^{-1}$ pepper essential oil	65–72%	[72]
<i>Monilinia laxa</i>	Brown Rot Blossom Blight and Twig Canker	<i>Citrus sinensis</i>	Crude extract	Complete inhibition	[73]

During reproductive phases, EOs inhibit the formation and germination of conidia, the asexual spores responsible for fungal dissemination [78]. Compounds such as thymol, carvacrol, and eugenol decrease spore size, viability, and germination rates, reducing infection potential [79]. Tea tree and cinnamon oils alter hyphal and mycelial morphology, suppressing aerial mycelium development and metabolic activity [80].

Similarly, neem (*Azadirachta indica*) and tea tree EOs significantly inhibit urediniospore germination in *Hemileia vastatrix*, demonstrating their potential as natural fungicides for agricultural systems [81].

4.4. Oxidative Stress and Lipid Peroxidation as Determinants of Antifungal Activity

One of the central antifungal mechanisms of EOs is the induction of oxidative stress through excessive generation of reactive oxygen species (ROS) [82]. When EO constituents penetrate fungal membranes, phenolic and terpene compounds disrupt mitochondrial cytochrome c oxidase activity, leading to electron leakage and one-electron reduction of oxygen to superoxide radicals ($\text{O}_2^{\cdot -}$) [83].

These radicals are subsequently converted to hydrogen peroxide (H_2O_2) by SOD and further transformed into hydroxyl radicals ($\bullet\text{OH}$) via Fenton reactions [84]. The accumulation of ROS overwhelms the fungal antioxidant defenses, resulting in oxidative damage to lipids, proteins, and nucleic acids [85].

Thymol and carvacrol are particularly effective in triggering ROS-mediated apoptosis-like pathways in *Candida albicans* and *Aspergillus niger* [86]. Similarly, cinnamaldehyde and eugenol raise intracellular ROS levels, causing lipid peroxidation, mitochondrial dysfunction, and membrane collapse [87].

Lipid peroxidation represents a critical downstream event following EO exposure, involving the oxidative degradation of polyunsaturated fatty acids within the fungal phospholipid bilayer. This reaction yields reactive lipid peroxides that propagate further oxidative damage [88], disrupt membrane permeability, and lead to cellular lysis [89].

Figure 3 illustrates the oxidative-stress cascade triggered by EO exposure, highlighting its role in reducing ATP production and compromising fungal viability.

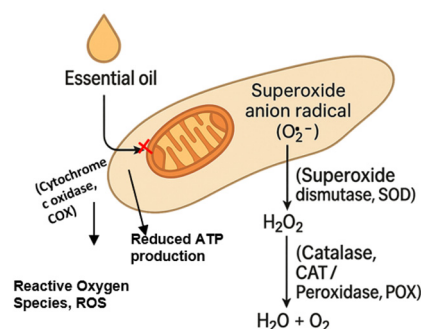


Figure 3. Essential oil-induced oxidative stress cascade in a fungal hypha. Contact with a plant essential oil (EO) inhibits mitochondrial cytochrome c oxidase (Complex IV), promoting electron leakage and the one-electron reduction of molecular oxygen to the superoxide anion radical ($O_2^{\cdot-}$). Superoxide dismutase (SOD) rapidly dismutates $O_2^{\cdot-}$ to hydrogen peroxide (H_2O_2), which is normally detoxified to water and molecular oxygen by catalase (CAT) and peroxidase (POX). Excessive ROS generation, together with the limited capacity of antioxidant enzymes, leads to the intracellular accumulation of $O_2^{\cdot-}$ and H_2O_2 , initiating oxidative damage to lipids, proteins and nucleic acids and thereby compromising fungal viability.

4.5. Technological, Formulation, and Regulatory Challenges in the Development of EO-Based Biofungicides for Field Application

While the biochemical and physiological mechanisms of essential oils (EOs) as antifungal agents are increasingly well characterized, their translation from laboratory assays to consistent field performance remains constrained by several technological and regulatory barriers [90]. Unlike synthetic fungicides, EO-based products face intrinsic physicochemical limitations such as high volatility, poor water solubility, and susceptibility to oxidation that significantly reduce their persistence under agricultural conditions [91,92]. These limitations hinder dose standardization and long-term disease suppression, especially in open-field environments exposed to fluctuating temperature, humidity, and UV radiation [93].

From a formulation standpoint, ensuring the controlled release and stability of EO constituents is one of the most critical technological challenges. Conventional emulsions and wettable powders often fail to protect the active compounds from rapid degradation or evaporation [94]. Consequently, research has increasingly focused on encapsulation and nanotechnology-based systems, such as liposomes, nanoemulsions, polymeric nanoparticles, and cyclodextrin complexes that enhance bioavailability, reduce volatility, and extend field efficacy [95]. Despite promising results in controlled environments, scalability and cost-efficiency remain limiting factors for large-scale production [96].

Furthermore, the regulatory framework for botanical biofungicides presents another barrier. Registration procedures for EO-based products are frequently adapted from chemical pesticide frameworks, which do not always account for the complex, multicomponent nature of EOs. This misalignment delays approval processes and constrains innovation in developing novel formulations [97]. Harmonized international guidelines that balance efficacy, safety, and sustainability are urgently needed to facilitate market integration.

As summarized in Table 5, these challenges encompass volatility, environmental degradation, formulation stability, production costs, regulatory constraints, and limited field validation. Addressing them requires interdisciplinary collaboration that integrates formulation science, nanotechnology, agronomy, and environmental toxicology. Such an integrated approach will bridge the current gap between laboratory innovation and

agricultural implementation, ultimately consolidating EO-based biofungicides as viable components of sustainable crop protection systems.

Table 5. Key limitations hindering development and field application of essential oil-based biofungicides.

Limitation	Consequences	Solution	Reference
Low persistence	Pathogens with rapid infection cycles can infect immediately after the EO concentration falls below the inhibitory level.	Nano/Micro-Encapsulation of EO droplets within a polymeric matrix (e.g., chitosan, alginate, lipids).	[98]
High volatility	Compounds like thymol and eugenol possess vapor pressures higher than conventional fungicides, driving rapid phase transition from liquid to vapor state.	Require synchronization with the plant pathogenic fungi biology. Re-engineering of delivery systems of EOs.	[99]
Photosensitivity	Susceptibility to solar radiation under field conditions creates a significant efficacy gap.	Use of natural radical scavengers (rosmarinic acid, tocopherols) that preferentially absorb damaging radiation.	[100]
Rapid degradation	Phyllosphere and soil microbiota rapidly metabolize EO components through enzymatic oxidation, reduction, and conjugation reactions.	Encapsulate the EO within a polymeric shell (chitosan or lignin-based products)	[101]
Low aqueous solubility	Hydrophobic EOs naturally separate from the water-based spray solutions.	Utilizing high-energy (ultrasonication, high-pressure homogenization) for producing nanoemulsion	[102]
Potential phytotoxicity	When dose is high, localized necrosis, generalized chlorosis, epinastic leaf deformation, and premature abscission of leaves may be observed.	Nano-formulations for targeted delivery and controlled release, coupled with the establishment of optimized application protocols based on crop-specific physiological windows and environmental conditions	[103]
Technique of application	Non-uniform coverage, large droplets, application in full sun, application before rain, timing in disease cycle of plant pathogenic fungi	Electrostatic sprayers or fine, ultra-low-volume (ULV) nozzles for uniform coverage, employing adjuvants to optimize droplet size and adherence, scheduling applications during early morning or late evening hours to minimize volatilization and UV degradation, leveraging disease forecasting models for precise timing relative to pathogen life cycles, and implementing water-sensitive paper for real-time monitoring of spray distribution and droplet quality	[104]
Cost	Low viability and limited commercial adoption for farmers due to high cost	Growing specific high-yield, fungicidal plants on farm and processing them into effective, though less refined, botanical extracts instead of pure essential oils	[105]
Scalability	The development of commercial biofungicide requires mass production of EOs with consistent quality standard.	An integrated platform, combining plant cell culture in advanced bioreactors with precision metabolic engineering, can industrialize the synthesis of key antifungal metabolites derived from essential oils.	[106]

The practical implementation of essential oils (EOs) as biofungicides requires careful consideration of their physicochemical behavior and ecological interactions. When applied at appropriate doses, EOs can effectively suppress phytopathogenic fungi without causing phytotoxic effects or compromising plant vitality. Their high volatility and low residuality make them environmentally benign when used with optimized application equipment and under suitable environmental conditions, ensuring safety and efficacy in the field. Conversely, excessive concentrations or repeated applications may induce phytotoxicity, underscoring the importance of dosage calibration and controlled formulations.

Furthermore, evidence indicates that EOs pose minimal risk to beneficial soil microbiota, as they are typically applied in low quantities and are rapidly degraded by soil microorganisms, including *Bacillus*, *Trichoderma*, and other beneficial fungi. These organisms can metabolize EO constituents as carbon sources, contributing to their detoxification and integration into natural biogeochemical cycles. Consequently, EOs demonstrate strong potential as eco-compatible biofungicides, balancing antifungal efficacy with environmental safety (Figure 4).

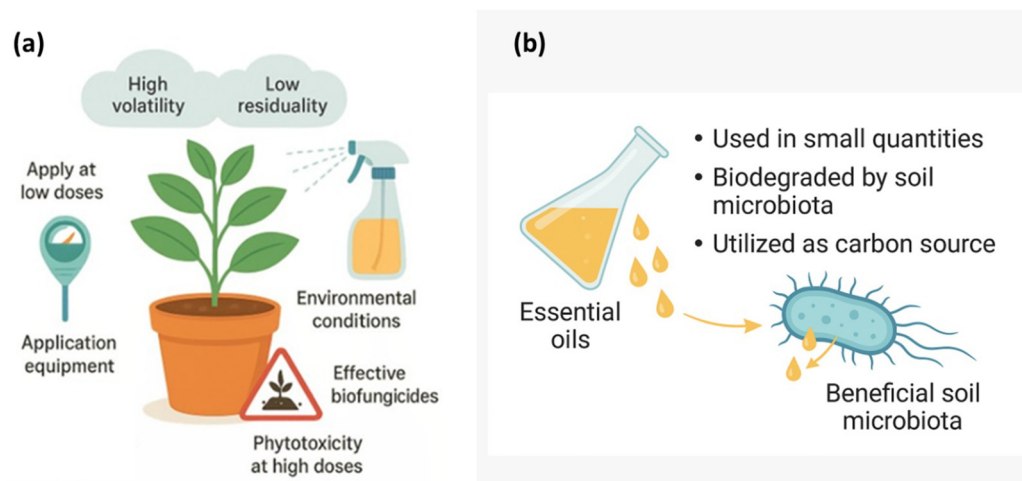


Figure 4. Safe application and environmental compatibility of essential oils as biofungicides. (a) Appropriate use of essential oils on plants under field conditions considering volatility, residuality, dosage, equipment, and environmental factors. (b) Interaction between essential oils and beneficial soil microbiota showing their biodegradation and utilization as a carbon source.

4.6. Technological Frontiers in EO-Based Biofungicide Development: From Extraction to Field Application

It is true that promising laboratory results with EOs sometimes fail to translate to field-effective bioproducts [107]. The critical bridge between fundamental knowledge and practical application lies in addressing three interconnected technological domains: advanced extraction methodologies [108], stabilization through innovative formulation [109], and precision delivery systems [110].

The chemical complexity of EOs, while central to their multi-target action, presents a primary challenge for standardization [111]. Modern extraction has evolved beyond conventional steam distillation. Supercritical fluid extraction (SFE), particularly using CO₂, has emerged as a superior technology [112]. SFE allows for precise modulation of temperature and pressure to selectively extract target bioactive compounds with higher yield and minimal thermal degradation, ensuring a more consistent and potent product [113].

Furthermore, the integration of metabolomic fingerprinting using GC-MS and LC-MS is becoming indispensable for quality control [114]. This is crucial for regulatory approval and for growers who require predictable performance [115]. For high-value compounds whose plant sources are rare or slow-growing, plant cell culture in bioreactors offers a

revolutionary, climate-independent production platform, ensuring supply chain stability and freeing production from agricultural constraints [116].

The inherent volatility, photosensitivity, and hydrophobicity of EOs have long been an important limitation in field conditions [117]. The most significant progress in the last five years has come from nano- and micro-technology. EO droplets within a polymeric matrix is no longer a novel concept, but the sophistication of these systems has dramatically increased [118]. Current research focuses on stimuli-responsive nanocarriers. For instance, chitosan-based nanocapsules can be engineered to release their payload in response to the specific enzymatic activity (e.g., cutinases, cellulases) of the target fungus at the infection site [119]. This targeted delivery minimizes non-target losses and prolongs antifungal activity. Similarly, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) provide superior physical stability and protection against UV degradation compared to traditional emulsions [120].

To combat photodegradation, the simple addition of natural, food-grade antioxidants like rosmarinic acid and tocopherols directly into the oil phase before encapsulation has proven highly effective [121]. These compounds act by absorbing damaging radiation and quenching free radicals, thereby protecting the bioactive terpenes and phenols [122]. This approach significantly narrows the efficacy gap between laboratory and field conditions.

Electrostatic sprayers that impart a negative charge to droplets ensure that they wrap around and adhere to both sides of plant leaves, drastically improving coverage and reducing drift [123]. Coupled with ultra-low-volume (ULV) nozzles, which produce a uniform fine mist, these systems ensure optimal deposition with minimal product volume [124].

Furthermore, application timing must be intelligent. Integrating EO sprays into decision support systems (DSSs) that utilize disease forecasting models [125] allows for prophylactic application just before critical infection periods [126]. This strategic timing maximizes the impact of the EO's protective action, disrupting the pathogen's life cycle at its most vulnerable stage.

5. Conclusions

Plant essential oils (EOs) are a relevant ecologically alternative for sustainable crop protection. Their multifaceted antifungal mechanisms including membrane disruption, enzyme inhibition, and induction of oxidative stress provide a robust, nature-derived arsenal with a broad spectrum to control devastating phytopathogenic fungi. However, the central thesis emerging from this review is that the primary barrier to their widespread adoption is no longer a question of efficacy, but one of translational science. The volatility, hydrophobicity, and chemical complexity of essential oil also give rise to critical challenges in photostability, field persistence, and phytotoxicity. Consequently, the historical focus on bio-prospecting and in vitro screening has reached a point of diminishing returns. The path forward demands a paradigm shift from discovery to innovation in formulation and delivery. The future of EO-based biofungicides hinges on the strategic convergence of advanced material science for nanoencapsulation, bioprocess engineering for the scalable production of standardized metabolites in bioreactors, and precision agriculture for targeted application. Next, the thematics of research must be fundamentally interdisciplinary. It is only through the synergistic integration of these fields that EOs can transcend their current status as promising laboratory candidates and emerge as reliable, cornerstone tools in next-generation Integrated Pest Management (IPM) systems. This integrated approach is the indispensable key to unlocking their full potential, creating a route for their transition from a niche alternative to a mainstream solution for building more resilient and sustainable global agriculture.

Author Contributions: Conceptualization, M.L.-M.; writing—original draft preparation, M.L.-M.; D.B.; C.A.; K.H.; D.G.-F.; O.L.-H.; L.R.S.; P.S.P. and A.B.; review and editing, M.L.-M.; and A.B. All authors have read and agreed to the published version of the manuscript.

Funding: We gratefully acknowledge the support of the Dirección de Investigación y Desarrollo of the Universidad Técnica de Ambato (UTA), as well as the contributions of the researchers and individuals involved in this project. Special thanks to the authorities of the Faculty of Agricultural Sciences and DIDE-UTA. This work was funded by Michel Leiva-Mora under the research project EVALUACIÓN DE LA ACTIVIDAD ANTIFÚNGICA DE ACEITES ESENCIALES MICROENCAPSULADOS MEDIANTE MÉTODOS DE DILUCIÓN EN AGAR Y RESARZURINA. (Resolución Nro. UTA-CONIN-2025-0046-R).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: The authors would like to thank the Dirección de Investigación y Desarrollo (DIDE) of the Universidad Técnica de Ambato for its administrative support.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

EOs	Essential Oils
ROS	Reactive Oxygen Species
IPM	Integrated Pest Management
TEO	Thyme Essential Oil
EC50	Effective Concentration 50 (concentration at which 50% of maximal effect is observed)
CHI	Chitinase
PPO	Polyphenol Oxidase
POX	Guaiacol Peroxidase
PAL	Phenylalanine Ammonia-Lyase
CcO	Cytochrome c Oxidase
NADH	Nicotinamide Adenine Dinucleotide (reduced form)
CAT	Catalase
SOD	Superoxide Dismutase
QS	Quorum Sensing
HMGR	3-hydroxy-3-methylglutaryl-CoA Reductase
HMG-CoA	3-hydroxy-3-methylglutaryl-Coenzyme A
PMK	Phosphomevalonate Kinase
MVD	Mevalonate-5-pyrophosphate Decarboxylase
IPP	Isopentenyl Pyrophosphate
FPPS	Farnesyl Pyrophosphate Synthase
FPP	Farnesyl Pyrophosphate
LSS	Lanosterol Synthase
CYP51	C-14 Sterol Demethylase (Cytochrome P450 51)
MIC	Minimum Inhibitory Concentration

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