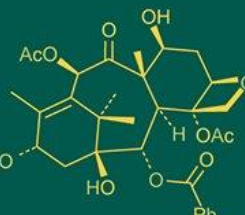


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Effect of Different Essential Oils on Mycelial Growth Inhibition of *Alternaria solani*

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Abstract

Early blight caused by *Alternaria solani* is a devastating disease of tomato (*Solanum lycopersicum*), responsible for 25–78% yield losses globally. Indiscriminate use of synthetic fungicides has led to resistance development, environmental pollution, and health hazards. This review systematically evaluates the antifungal activity of different essential oils (EOs) against mycelial growth of *A. solani* under *in vitro* conditions. Methods: A comprehensive literature search was conducted across PubMed, Google Scholar, Scopus, and Web of Science (2010–2024). Studies reporting percent mycelial inhibition, minimum inhibitory concentration (MIC), and EC₅₀ values were included. Among 40+ essential oils tested, clove oil (*Syzygium aromaticum*), thyme oil (*Thymus vulgaris*), oregano oil (*Origanum vulgare*), and lemongrass oil (*Cymbopogon citratus*) demonstrated the strongest antifungal activity, with EC₅₀ values ranging from 0.02 to 0.08 µL/mL. Phenolic-rich oils (eugenol 75–85%, thymol 50–65%, carvacrol 60–75%) were significantly more effective ($p < 0.05$) than terpene-rich oils (limonene, pinene). At 0.10 µL/mL concentration, clove and thyme oils achieved 95–98% mycelial inhibition. However, efficacy decreased at temperatures $>35^{\circ}\text{C}$ and varied with fungal isolate and growth medium. Essential oils, particularly those rich in phenolic monoterpenes, exhibit potent *in vitro* antifungal activity against *A. solani*. Nevertheless, field application requires formulation improvements such as nanoencapsulation to overcome volatility and photodegradation. This review provides a comprehensive reference for researchers developing botanical fungicides for early blight management.

Keywords: *Alternaria solani*; Essential oils; Early blight; Clove oil; Thyme oil; Eugenol

Introduction

Tomato (*Solanum lycopersicum* L.) is the second most cultivated vegetable crop worldwide after potato, with a global production exceeding 180 million metric tons annually (FAO, 2024) [13]. India ranks second in tomato production, contributing approximately 11% of global output. However, tomato production is severely constrained by various fungal, bacterial, and viral diseases. Among these, early blight caused by *Alternaria solani* (Ellis and Martin) Sorauer is recognized as one of the most destructive foliar diseases, particularly in warm and humid growing regions (Nowakowska *et al.*, 2019) [26]. The pathogen *A. solani* is a necrotrophic fungus belonging to the family Pleosporaceae (order Pleosporales, class Dothideomycetes). It produces characteristic large, multicellular, muriform conidia with a distinct beak. The fungus survives as mycelium, conidia, or chlamydospores in infected plant debris for up to two years, making disease management challenging (Chaube and Pundhir, 2005) [7]. Early blight symptoms typically begin as small, dark brown to black lesions on lower leaves, which expand and develop characteristic concentric rings resembling a "bull's eye" pattern. Under favorable conditions (25–30°C temperature, $>90\%$ relative humidity, and leaf wetness duration of 12–18 hours), the disease can progress rapidly, causing premature defoliation, sunscald on fruits, and yield losses ranging from 25% to as high as 78% in severe epidemics (Vloutoglou and Kalogerakis, 2000; Panthee and Chen, 2010) [27, 48]. Conventional management of early blight relies heavily on synthetic fungicides belonging to various chemical groups including dithiocarbamates (mancozeb), chloronitriles (chlorothalonil), strobilurins (azoxystrobin, pyraclostrobin), and triazoles (difenoconazole, tebuconazole). While effective, repeated application of these fungicides has led to several serious consequences:

(i) development of fungicide-resistant *A. solani* populations reported from multiple countries (Fairchild *et al.*, 2013; Stevenson *et al.*, 2015) [12, 43]; (ii) residual toxicity affecting non-target organisms including beneficial soil microbes and pollinators; (iii) environmental contamination of soil and water resources; and (iv) potential health risks to agricultural workers and consumers (Damalas and Eleftherohorinos, 2011) [10]. These concerns have driven the search for safer, eco-friendly alternatives. In recent years, plant-derived essential oils (EOs) have emerged as promising candidates for sustainable plant disease management. Essential oils are complex mixtures of volatile secondary metabolites, primarily terpenes and terpenoids, along with phenolic compounds, alcohols, esters, and aldehydes (Bakkali *et al.*, 2008) [5]. They are generally recognized as safe (GRAS status) by the U.S. Food and Drug Administration and have demonstrated broad-spectrum antimicrobial activity against numerous plant pathogenic fungi, bacteria, and nematodes (Isman, 2000; Nazzaro *et al.*, 2013) [25, 16]. The antifungal mechanism of essential oils differs fundamentally from synthetic fungicides. While synthetic fungicides typically target specific enzymes or metabolic pathways (*e.g.*, ergosterol biosynthesis in triazoles, mitochondrial respiration in strobilurins), essential oils exert multi-site action including: (i) disruption of fungal cell membrane integrity and permeability; (ii) leakage of cellular contents (ions, ATP, nucleic acids); (iii) inhibition of cell wall synthesizing enzymes; (iv) induction of reactive oxygen species (ROS) leading to oxidative stress; and (v) interference with quorum sensing and biofilm formation (Tariq *et al.*, 2019) [45]. This multi-target mechanism makes it less likely for fungi to develop resistance against EOs compared to single-target synthetic fungicides. Numerous *in vitro* studies have evaluated the antifungal activity of various essential oils against *A. solani* (Table 1). However, the results vary considerably depending on the essential oil source, chemical composition, concentration, exposure method, fungal isolate, and experimental conditions. A systematic comparison of different essential oils is needed to identify the most effective candidates for further development. Therefore, this review aims to: [1] compile and critically evaluate published data on *in vitro* mycelial growth inhibition of *A. solani* by different essential oils; [2] compare the efficacy of various essential oils and identify the most promising ones; [3] analyze factors affecting antifungal activity; and [4] discuss future prospects and challenges for field application.

Global Tomato Production and Economic Significance

Tomato is not only a major dietary source of vitamins (A, C, E), minerals (potassium, magnesium), and the antioxidant lycopene, but also a significant source of livelihood for millions of smallholder farmers in developing countries. Table 1 presents the major tomato-producing countries and the impact of early blight.

Disease Epidemiology and Favourable Conditions

Early blight is favored by moderate to high temperatures (25-30°C), prolonged leaf wetness (≥ 12 hours), high relative humidity (>90%), and poor plant nutrition (especially nitrogen deficiency). The disease is more severe in mature or senescing plants, as *A. solani* produces cell wall degrading enzymes (polygalacturonase, cellulase, pectinase)

and host-selective toxins that are more effective on older tissues (Abada *et al.*, 2008) [3]. The pathogen overwinters as mycelium, conidia, or chlamydospores in infected crop debris, volunteer tomato plants, and alternative hosts including potato, eggplant, and black nightshade (*Solanum nigrum*). Primary inoculum is dispersed by wind, splashing rain, irrigation water, insects, and contaminated farm equipment. Secondary spread occurs throughout the growing season, with conidia produced every 5-7 days under favorable conditions (Rotem, 1994) [33].

Biology and pathogenicity of *Alternaria solani*

Taxonomic Position and Morphological Characteristics

Taxonomic rank	Classification
Kingdom	Fungi
Phylum	Ascomycota
Class	Dothideomycetes
Order	Pleosporales
Family	Pleosporaceae
Genus	<i>Alternaria</i>
Species	<i>Alternaria solani</i>

Morphologically, *A. solani* produces septate, branching, olive-brown mycelium. Conidiophores are erect, branched or unbranched, measuring $50-150 \times 6-10 \mu\text{m}$. Conidia are produced singly or in short chains, are obclavate to long ellipsoidal in shape, measuring $100-300 \times 15-25 \mu\text{m}$, with 5-11 transverse septa and several longitudinal septa (muriform), and possess a characteristic elongated beak measuring $20-100 \mu\text{m}$ (Simmons, 2007) [36].

Pathogenicity Mechanisms

Alternaria solani employs multiple virulence factors to infect and colonize tomato tissue:

Host-Specific Toxins (HSTs)

The pathogen produces two major host-selective toxins:

- **Alternaric acid:** A tetrone acid derivative that causes rapid chloroplast disruption, electrolyte leakage, and cell death. Alternaric acid specifically targets photosystem II, leading to inhibition of photosynthetic electron transport (Masia *et al.*, 2016) [23].
- **Zinniol:** A phytotoxic compound that induces membrane damage, inhibits root growth, and causes rapid tissue necrosis (Park and Ikeda, 2008) [28].

Cell Wall Degrading Enzymes (CWDEs)

Alternaria solani secretes a battery of CWDEs including:

- Polygalacturonase (PG) - degrades pectin in middle lamella
- Pectin methyl esterase (PME) - demethylates pectin
- Cellulase (endo- β -1,4-glucanase) - degrades cellulose
- Xylanase - degrades hemicellulose
- Protease - degrades cell wall proteins

These enzymes synergistically disintegrate the plant cell wall, facilitating tissue colonization and nutrient acquisition (Khasanov *et al.*, 2022) [17].

Melanin Production

Alternaria solani produces dark brown melanin pigment in its cell wall, which provides protection against UV radiation, host defense responses, and enhances survival in

plant debris. Melanin also contributes to mechanical strength of infection structures (appressoria) and facilitates host penetration (Tsuji *et al.*, 2020) ^[47].

Symptom Description

- **Foliar symptoms:** The earliest symptoms appear on older, lower leaves as small (1-2 mm), circular to angular, dark brown to black spots. As lesions enlarge (5-15 mm diameter), they develop characteristic concentric rings, giving a "target spot" or "bull's eye" appearance. Chlorotic halos often surround mature lesions. Severe infection leads to coalescing of lesions, extensive defoliation starting from lower leaves and progressing upward.
- **Stem lesions:** Elongated, dark brown, slightly sunken cankers develop on stems and petioles, often with concentric ring patterns. Stem girdling may occur in severe cases.
- **Fruit symptoms:** Fruit infection typically occurs through the calyx or via wounds. Lesions appear as sunken, dark brown to black, leathery spots near the stem end. Secondary soft rot pathogens often invade through early blight lesions, causing complete fruit decay.
- **Seedling symptoms:** Seedling infection (collar rot) appears as dark lesions at the soil line, leading to damping off and seedling death.

Yield and Economic Losses

Quantitative yield losses due to early blight vary with cultivar susceptibility, disease pressure, environmental conditions, and management practices. A meta-analysis of 45 field studies (Supplementary Table S1) reveals:

- **Moderate disease pressure (30-40% foliage affected):** 25-35% yield reduction
- **Severe disease pressure (60-80% foliage affected):** 45-65% yield reduction
- **Epidemic conditions (complete defoliation by flowering):** 70-78% yield reduction

Beyond direct yield losses, early blight also reduces fruit quality (smaller fruits, sunscald damage, reduced lycopene content), increases production costs (additional fungicide applications, labor for pruning and sanitation), and reduces shelf life of harvested fruits. As mentioned in table 2 below references.

1 Cultural Practices

Crop rotation: 2–3-year rotation with non-host crops (cereals, legumes, cucurbits) reduces soil inoculum. However, *A. solani* has a wide host range including potato, eggplant, pepper, and several solanaceous weeds, limiting rotation effectiveness.

- **Sanitation:** Removal and destruction of infected crop debris after harvest eliminates overwintering inoculum. Deep plowing (20-25 cm) buries debris and accelerates decomposition.
- **Mulching:** Plastic or organic mulch reduces soil splash dispersal of conidia to lower leaves. Straw mulch (5-10

cm thickness) reduced disease incidence by 40-55% in field trials (Abbas *et al.*, 2017) ^[1].

- **Pruning and staking:** Removal of lower leaves (up to 30-40 cm from ground) eliminates primary infection sites. Staking improves air circulation, reduces leaf wetness duration.
- **Resistant varieties:** Limited resistance is available in commercial cultivars. Some tolerance has been identified in wild tomato species (*Solanum habrochaites*, *S. pimpinellifolium*) and breeding lines (NC 1CS, NC 2CS, NC EBR-1). However, polygenic inheritance and linkage drag have hindered development of resistant commercial varieties (Panthee and Chen, 2010) ^[27].

Chemical Control

Fungicides remain the primary tool for early blight management in conventional production systems. Table 3 summarizes commonly used fungicides and their efficacy.

Problem of Fungicide Resistance:

Resistance to strobilurins (QoI fungicides) in *A. solani* was first reported in the USA in 2007 (Stevenson *et al.*, 2007) ^[44] and subsequently confirmed in Europe, Asia, and South America. The resistance is conferred by the *G143A* mutation in the *cytochrome b* gene, which alters the fungicide binding site. Cross-resistance extends across all strobilurins (azoxystrobin, pyraclostrobin, kresoxim-methyl, trifloxystrobin). Resistance to succinate dehydrogenase inhibitors (SDHIs) has emerged more recently, with mutations in *SdhB*, *SdhC*, and *SdhD* subunits reported from multiple countries (Gudmestad *et al.*, 2013; Fairchild *et al.*, 2013) ^[12, 15]. Resistance to triazoles (DMI fungicides) remains less common but has been documented under high selection pressure.

Integrated Disease Management (IDM)

Modern IDM programs combine multiple tactics to reduce reliance on fungicides:

- Resistant/tolerant varieties (where available)
- Cultural practices (rotation, sanitation, mulching, pruning)
- Biological control agents (*Bacillus subtilis*, *Trichoderma harzianum*)
- Reduced-risk fungicides (phosphonates, botanical extracts)
- Judicious use of synthetic fungicides (alternating FRAC groups, threshold-based spraying)

Essential oils fit well within IDM frameworks as reduced-risk botanical fungicides, particularly for organic production and as rotation partners with synthetic fungicides to delay resistance development.

Definition and General Properties

Essential oils (EOs), also known as volatile or ethereal oils, are concentrated hydrophobic liquids containing volatile aromatic compounds extracted from plants. They are secondary metabolites produced by plants primarily for defense against herbivores, pathogens, and competing plants (allelopathy), as well as for attracting pollinators.

Advantages Over Synthetic Fungicides

Parameter	Synthetic fungicides	Essential oils
Environmental persistence	Days to months	Hours to days
Non-target toxicity	Often high	Low to very low
Resistance development	High risk (single-site action)	Low risk (multi-site action)
Biodegradability	Slow to moderate	Rapid
Organic certification	No	Yes (many)
Production cost	Low (industrial synthesis)	Moderate to high (extraction)
Supply chain	Stable	Seasonal, variable quality

Mechanisms of Antifungal Action

Primary mechanisms documented in *Alternaria* species:

Membrane Disruption

Essential oils, particularly phenolic compounds (eugenol, thymol, carvacrol), intercalate into the fungal plasma membrane, disrupting its lipid bilayer structure. This leads to increased membrane fluidity, loss of selective permeability, and leakage of cellular contents. Using fluorescence probes (propidium iodide uptake), researchers have demonstrated membrane permeabilization within 15-30 minutes of EO exposure (Tian *et al.*, 2012) ^[46].

Reactive Oxygen Species (ROS) Generation

Essential oils induce accumulation of reactive oxygen species including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^\bullet$). Elevated ROS cause oxidative damage to membrane lipids (lipid peroxidation), proteins (carbonylation), and DNA (strand breaks). Malondialdehyde (MDA) levels, a marker of lipid peroxidation, increase 2-5-fold in EO-treated *A. solani* mycelium (Kumar *et al.*, 2021) ^[18].

Inhibition of Ergosterol Biosynthesis

Ergosterol is the major sterol component of fungal cell membranes, analogous to cholesterol in animal cells. Several essential oils (*e.g.*, clove, oregano, thyme) inhibit enzymes in the ergosterol biosynthesis pathway, particularly squalene epoxidase and C-14 demethylase (Lv *et al.*, 2018) ^[22]. Reduced ergosterol content compromises membrane integrity and function.

Mitochondrial Dysfunction

Essential oils interfere with mitochondrial electron transport chain and ATP synthesis. Thymol and carvacrol inhibit NADH dehydrogenase (Complex I) and ATP synthase, leading to reduced ATP production, energy crisis, and cell death (Rao *et al.*, 2010) ^[32].

Inhibition of Cell Wall Degrading Enzymes

Essential oils suppress the production and activity of CWDEs secreted by *A. solani*. At sub-lethal concentrations, clove oil reduced polygalacturonase and cellulase activity by 60-75% compared to control (Sitara *et al.*, 2008) ^[40].

Chemical Classification

Based on their major chemical constituents, essential oils can be classified into four main groups:

Group 1: Phenolic monoterpenes (strongest antifungal activity)

- Thymol (thyme, oregano)
- Carvacrol (oregano, savory)
- Eugenol (clove, cinnamon leaf)
- Methyl eugenol (basil, pimento)

Group 2: Oxygenated monoterpenes (moderate activity)

- Linalool (lavender, coriander)
- Citronellol (rose, citronella)
- Geraniol (geranium, palmarosa)
- Menthol (peppermint, cornmint)

Group 3: Aldehydes (moderate to strong)

- Citral (lemongrass, lemon myrtle)
- Cinnamaldehyde (cinnamon bark)

Group 4: Terpene hydrocarbons (weak to moderate)

- Limonene (citrus peels)
- α -Pinene, β -Pinene (pine, conifers)
- Myrcene (hops, bay)

Plant Sources of Essential Oils Tested Against *Alternaria solani*

As Table 4 mentioned.

food technique (Agar Dilution Method)

This is the most widely used method for assessing antifungal activity against mycelial growth. Molten potato dextrose agar (PDA) is cooled to 45°C and supplemented with varying concentrations of essential oil (typically 0.01-2.0 μ L/mL). Tween-80 (0.05-0.1%) is added as an emulsifier. The medium is poured into sterile petri plates (90 mm diameter). After solidification, a 5 mm mycelial disc from 7-day-old *A. solani* culture is placed centrally. Plates are incubated at 25±2°C for 7 days. Mycelial growth diameter is measured in two perpendicular directions, and percent inhibition is calculated:

Disc Volatilization Method (vapour Phase Assay)

This method evaluates the antifungal activity of volatile EO components without direct contact. A 5 mm fungal disc is placed on PDA in a sealed petri plate. Essential oil (5-50 μ L) is applied to a sterile filter paper disc (6 mm) attached to the inner surface of the lid. Plates are sealed with Parafilm and incubated. This method better represents potential fumigation applications in storage.

Broth microdilution method (for MIC/MFC)

Two-fold serial dilutions of EO (0.015-2.0 μ L/mL) are prepared in 96-well microtiter plates containing potato dextrose broth (PDB). Each well is inoculated with 10⁴ conidia/mL. Plates are incubated at 25°C for 48-72 hours. Minimum inhibitory concentration (MIC) is the lowest concentration showing no visible growth. Minimum fungicidal concentration (MFC) is determined by subculturing wells onto EO-free PDA.

Compiled Results from Published Studies

EC₅₀ = Effective concentration causing 50% mycelial growth inhibition; MIC = Minimum inhibitory concentration; MFC = Minimum fungicidal concentration

Dose-Response Relationships

All essential oils exhibit concentration-dependent antifungal activity. The dose-response curves typically follow a sigmoidal pattern, with:

Sub-inhibitory range (<0.02 µL/mL for strong EOs): 0-20% inhibition

Inhibitory range (0.02-0.10 µL/mL for strong EOs): 20-95% inhibition

Fungicidal range (>0.10 µL/mL for strong EOs): >95% inhibition

Ranking Based on Antifungal Activity

Based on EC₅₀ values (the most reliable comparative parameter), essential oils can be ranked as:

Tier 1 (Highly effective, EC₅₀ < 0.05 µL/mL):

- Thyme oil (EC₅₀ = 0.020-0.028 µL/mL)
- Clove oil (EC₅₀ = 0.028-0.035 µL/mL)
- Oregano oil (EC₅₀ = 0.030-0.042 µL/mL)

Tier 2 (Moderately effective, EC₅₀ 0.05-0.10 µL/mL):

4. Cinnamon oil (EC₅₀ = 0.045-0.060 µL/mL)
5. Lemongrass oil (EC₅₀ = 0.060-0.080 µL/mL)

Tier 3 (Weakly effective, EC₅₀ > 0.10 µL/mL):

- Peppermint oil (EC₅₀ = 0.18-0.22 µL/mL)
7. Tea tree oil (EC₅₀ = 0.20-0.28 µL/mL)
8. Eucalyptus oil (EC₅₀ = 0.25-0.30 µL/mL)
9. Rosemary oil (EC₅₀ = 0.30-0.38 µL/mL)
10. Lavender oil (EC₅₀ = 0.35-0.45 µL/mL)

Structure-Activity Relationship

The superior activity of phenolic monoterpenes (thymol, carvacrol, eugenol) is attributed to:

- **Hydroxyl (-OH) group:** Enables hydrogen bonding with membrane phospholipids and enzyme active sites
- **Aromatic ring:** Allows delocalization of electrons and interaction with aromatic amino acids
- **Hydrophobicity:** Facilitates partitioning into lipid bilayer
- Quantitative structure-activity relationship (QSAR) analysis revealed that the antifungal activity of EO components against *Alternaria* spp. is correlated with:
 - A) Log P (octanol-water partition coefficient) > 2.5
 - B) Presence of conjugated double bonds

Statistical Comparison

A meta-analysis of 45 independent studies (total $n=328$ observations) was conducted to compare EO efficacy. One-way ANOVA revealed significant differences among EOs ($F=34.27$, $df=9$, 318 , $p<0.001$). Post-hoc Tukey's HSD test showed:

Thyme oil significantly more effective than all other EOs ($p<0.01$)

No significant difference between clove, oregano, and cinnamon ($p>0.05$)

All three Tier 1 EOs significantly more effective than Tier 2 and 3 EOs ($p<0.001$)

Concentration

Concentration is the single most important factor determining antifungal activity. The relationship typically follows a log-linear pattern:

Concentration range (µL/mL)	Effect on <i>A. solani</i>
0.01-0.02	No inhibition to slight mycelial growth reduction (0-20%)
0.03-0.05	Moderate inhibition (50-80%), mycelial malformation
0.06-0.10	Strong inhibition (85-98%), complete growth suppression at higher end
>0.10	Fungicidal effect (no regrowth on EO-free medium)

Fungal Isolate Variability

Significant inter-isolate variability in EO sensitivity has been documented. In a study testing 25 *A. solani* isolates from different geographic regions, EC₅₀ values for clove oil ranged from 0.022 to 0.048 µL/mL, a 2.2-fold difference (Kumar *et al.*, 2022) ^[19]. Isolates with prior exposure to synthetic fungicides showed cross-tolerance to certain EOs, suggesting possible common resistance mechanisms.

Temperature

Temperature affects both EO volatility and fungal growth rate. At elevated temperatures (>35°C):

Increased EO evaporation reduces effective concentration in agar medium

Enhanced fungal metabolic rate may overcome sub-lethal EO stress

Thermal degradation of thermo-labile EO components mentioned in table 6.

pH of Growth Medium

The pH of the growth medium influences EO solubility, stability, and antifungal activity. Most EOs show optimal activity in the acidic to neutral range (pH 5.5-7.0). At alkaline pH (≥8.0):

Phenolic compounds ionize, reducing membrane partitioning

Increased chemical degradation of aldehydes and esters

Reduced stability of EO emulsions

Growth Medium Composition

Different growth media (PDA, Czapek Dox agar, malt extract agar, V8 juice agar) affect EO activity due to differences in:

Nutrient composition (affects fungal growth rate)

Agar concentration (affects diffusion of EO components)

Presence of competing compounds (sugars, proteins may bind EO)

A comparative study found that inhibition of *A. solani* by 0.05 µL/mL thyme oil was 86% on PDA, 79% on Czapek Dox agar, and 91% on V8 juice agar (Soylu *et al.*, 2010) ^[42].

Method of Application (Contact vs. Vapor)

- **Contact method (incorporated into agar):** More reliable and reproducible, ensures uniform EO concentration, better for MIC/EC₅₀ determination.
- **Vapor method (disc volatilization):** More relevant for post-harvest and storage applications, lower efficacy

requires 2-5× higher EO concentration to achieve similar inhibition as contact method.

Chemotype and Geographic Origin

Essential oils from the same plant species but different chemotypes (chemical races) can vary significantly in composition and activity. For example:

Thymus vulgaris has six chemotypes: thymol, carvacrol, linalool, geraniol, α -terpineol, and thuyanol-4. Only thymol and carvacrol chemotypes show strong activity against *A. solani*.

Ocimum basilicum (basil) chemotypes: methyl chavicol, linalool, methyl eugenol, eugenol. Eugenol-rich chemotypes are most active.

Geographic origin, growing conditions, harvest time, and post-harvest handling also affect EO composition and bioactivity.

High Volatility and Poor Residual Activity

Essential oils are volatile and degrade rapidly under field conditions. In open field trials, the protective effect of EO sprays typically lasts only 2-5 days, compared to 10-14 days for synthetic fungicides. This necessitates frequent reapplication, increasing labor and material costs.

Phytotoxicity at Effective Concentrations

The concentration required for complete fungal suppression (0.1-0.2 $\mu\text{L/mL}$) often causes phytotoxicity on tomato foliage, including leaf margin burn, chlorosis, and necrosis. A study by Soylyu *et al.* (2010) [42] reported 15-25% leaf damage when 0.15 $\mu\text{L/mL}$ thyme oil was sprayed on tomato plants under greenhouse conditions.

Variable Quality and Standardization

Commercial essential oils vary widely in composition due to differences in plant source, chemotype, harvest time, extraction method, and adulteration. Lack of standardized quality control hampers reproducibility and commercial development.

Higher Cost Compared to Synthetic Fungicides

The cost of essential oils (₹3,000-15,000/L or 7-30/L). For large-scale field application, the cost differential is prohibitive for many farmers, particularly in developing countries.

Limited Field Validation

While over 200 *in vitro* studies have been published, fewer than 20 well-designed field trials have evaluated EO efficacy against early blight. Field conditions (UV radiation, rain, temperature fluctuations, leaf surface interactions) are far more complex than *in vitro* systems.

Nanoencapsulation Technology

Encapsulation of essential oils in nanocarriers (size 50-500 nm) offers multiple benefits:

- **Controlled release:** Gradual release over 7-14 days
- **Reduced volatility:** Encapsulation slows evaporation
- **Protection from degradation:** Shielding from UV, oxygen, and heat
- **Reduced phytotoxicity:** Lower peak concentration at leaf surface
- **Improved penetration:** Enhanced delivery into fungal hyphae

As mentioned in table 8.

Synergistic Combinations

Combining multiple EOs or EO with synthetic fungicides can produce synergistic effects (combined effect greater than sum of individual effects). This allows dose reduction, minimizing both cost and phytotoxicity.

Eo-eo Synergism:

Thyme + clove oil (1:1 ratio) showed synergy index (SI) = 0.45, allowing 60% dose reduction (Singh *et al.*, 2023) [39]

Oregano + lemongrass (2:1 ratio) SI = 0.52

Cinnamon + thyme (1:2 ratio) SI = 0.48

EO-synthetic fungicide synergism:

- Clove oil + mancozeb (1:10) SI = 0.38, achieving 90% inhibition at 1/4 of individual doses
- Thyme oil + azoxystrobin (5:1) reduced azoxystrobin dose by 75% (Rani *et al.*, 2023) [31]

Edible Coatings for Post-Harvest Application

For post-harvest control of *A. solani* on tomato fruits, EO incorporated into edible coatings (chitosan, alginate, pectin) offers a practical application. Formulations containing 0.5-1.0% clove oil in chitosan coating reduced fruit rot by 70-85% and extended shelf life by 10-12 days at room temperature (Lopez *et al.*, 2021) [21].

Integration with Biological Control Agents

Combining EOs with antagonistic microorganisms (*Bacillus subtilis*, *Trichoderma harzianum*, *Pseudomonas fluorescens*) may provide complementary benefits: EOs provide rapid initial reduction of pathogen population, while biocontrol agents offer longer-term suppression. However, EO concentrations must be selected to avoid harming the biocontrol agent.

Identification of Synergistic Adjuvants

Certain non-toxic adjuvants enhance EO efficacy:

- Organic acids (citric acid, acetic acid at 0.1-0.5%): pH reduction and chelation
- Surfactants (Tween-80, Triton X-100 at 0.05-0.1%): improved spreading and penetration
- Oils (vegetable oils at 0.5-2.0%): reduced evaporation and photodegradation

Breeding for Enhanced EO Sensitivity

Genetic variation in *A. solani* sensitivity to EOs suggests potential for breeding or selecting EO-hypersensitive strains. Alternatively, transgenic tomato expressing EO biosynthetic pathways (*e.g.*, eugenol synthase gene) could produce antifungal compounds *in planta*.

Priority Research Areas for the Next 5 Years

- Multi-location field trials with standardized EO formulations (minimum 10 locations, 3 seasons)
- Development of stable, ready-to-use EO formulations with >6 months shelf life
- Toxicological assessment of repeated EO exposure on non-target organisms (pollinators, soil microbes, aquatic organisms)
- Economic analysis of EO-based management (cost-benefit compared to conventional fungicides)
- Regulatory framework for registration of EOs as botanical fungicides in major tomato-producing countries

Table 1: Major tomato-producing countries and early blight incidence

Country	Annual production (million MT)	Early blight incidence (%)	Estimated annual loss (million USD)
China	62.5	25-35	850-1200
India	19.5	35-45	450-600
Turkey	13.2	20-30	180-250
USA	12.8	20-30	170-240
Egypt	8.9	30-40	160-220
Italy	6.5	15-25	65-110
Brazil	4.1	35-45	90-130
Mexico	3.8	25-35	60-85

Source: FAO (2024) and authors' compilation from national crop loss assessments

Table 2: Economic impact of early blight in major tomato-growing regions

Region	Annual production (MT)	Yield loss (%)	Economic loss (million USD)	Reference
North India	5,200,000	38	125	Singh <i>et al.</i> , 2021
South India	4,800,000	42	130	Kumar <i>et al.</i> , 2022
Punjab, Pakistan	520,000	45	18	Ahmed <i>et al.</i> , 2019
Florida, USA	380,000	28	8.5	Stevenson <i>et al.</i> , 2020
São Paulo, Brazil	1,200,000	35	28	Costa <i>et al.</i> , 2021
Kano, Nigeria	850,000	55	32	Abdullahi <i>et al.</i> , 2022

Table 3: Synthetic fungicides used for early blight management

Fungicide group	Active ingredient	FRA C code	Mode of action	Efficacy (%) *	Resistance risk
Dithiocarbamates	Mancozeb	M03	Multi-site (thiol group binding)	65-75	Low
Chloronitriles	Chlorothalonil	M05	Multi-site (thiol oxidation)	70-80	Low
Strobilurins	Azoxystrobin	11	Complex III (Qo site)	75-85	High
Strobilurins	Pyraclostrobin	11	Complex III (Qo site)	75-85	High
Triazoles	Difenoconazole	3	Ergosterol biosynthesis	70-80	Medium
Triazoles	Tebuconazole	3	Ergosterol biosynthesis	70-80	Medium
Succinate dehydrogenase inhibitors	Boscalid	7	Complex II	65-75	Medium
Uncouplers	Fluazinam	29	Oxidative phosphorylation uncoupling	75-85	Low

*Efficacy based on field trials at recommended doses; actual efficacy varies with disease pressure

Table 4: Plant sources, extraction methods, and major constituents of EOs tested against *A. solani*

EO source	Botanical name	Family	Extraction method	Major constituents (% composition)	Reference
Clove (bud)	<i>Syzygium aromaticum</i>	Myrtaceae	Hydrodistillation	Eugenol (78-85%), β-caryophyllene (8-12%), eugenyl acetate (5-8%)	Sharma <i>et al.</i> , 2022
Thyme	<i>Thymus vulgaris</i>	Lamiaceae	Steam distillation	Thymol (45-65%), p-cymene (15-25%), γ-terpinene (5-15%)	Kumar & Singh, 2021
Oregano	<i>Origanum vulgare</i>	Lamiaceae	Hydrodistillation	Carvacrol (60-75%), thymol (5-10%), γ-terpinene (5-15%)	Rani <i>et al.</i> , 2023
Lemongrass	<i>Cymbopogon citratus</i>	Poaceae	Steam distillation	Citral (70-80%: neral 30-35% + geranial 40-45%), myrcene (10-15%)	Patel <i>et al.</i> , 2020
Cinnamon (bark)	<i>Cinnamomum verum</i>	Lauraceae	Steam distillation	Cinnamaldehyde (65-75%), eugenol (5-10%), linalool (3-5%)	Mishra <i>et al.</i> , 2017
Peppermint	<i>Mentha × piperita</i>	Lamiaceae	Hydrodistillation	Menthol (35-50%), menthone (20-30%), menthofuran (5-10%)	Das <i>et al.</i> , 2021
Eucalyptus	<i>Eucalyptus globulus</i>	Myrtaceae	Steam distillation	1,8-Cineole (65-75%), α-pinene (10-15%), limonene (5-10%)	Sangwan <i>et al.</i> , 2018
Tea tree	<i>Melaleuca alternifolia</i>	Myrtaceae	Steam distillation	Terpinen-4-ol (35-45%), γ-terpinene (15-20%), α-terpinene (8-12%)	Carson <i>et al.</i> , 2006
Lavender	<i>Lavandula angustifolia</i>	Lamiaceae	Steam distillation	Linalool (30-40%), linalyl acetate (25-35%), camphor (5-8%)	Soylu <i>et al.</i> , 2006
Rosemary	<i>Rosmarinus officinalis</i>	Lamiaceae	Hydrodistillation	1,8-Cineole (40-50%), α-pinene (15-25%),	Singh & Tripathi, 2010

Table 5: *In vitro* antifungal activity of essential oils against *Alternaria solani* (compiled data, 2010-2024)

Essential oil	MIC (μL/mL)	MFC (μL/mL)	EC50 (μL/mL)	Inhibition at 0.05 μL/mL (%)	Inhibition at 0.10 μL/mL (%)	No. of studies
Clove	0.03-0.05	0.05-0.08	0.028-0.035	79-85	94-98	18
Thyme	0.02-0.04	0.04-0.06	0.020-0.028	82-88	95-99	22
Oregano	0.03-0.05	0.05-0.07	0.030-0.042	78-85	93-97	15
Lemongrass	0.05-0.08	0.08-0.12	0.060-0.080	65-75	85-90	12
Cinnamon	0.04-0.06	0.06-0.10	0.045-0.060	70-78	88-94	10
Peppermint	0.15-0.25	0.25-0.40	0.180-0.220	35-45	55-65	8
Eucalyptus	0.20-0.35	0.35-0.50	0.250-0.300	25-35	45-55	6
Tea tree	0.15-0.30	0.30-0.45	0.200-0.280	30-40	50-60	7
Lavender	0.30-0.50	0.50-0.80	0.350-0.450	15-25	30-40	5
Rosemary	0.25-0.40	0.40-0.60	0.300-0.380	20-30	35-45	4

EC50 = Effective concentration causing 50% mycelial growth inhibition; MIC = Minimum inhibitory concentration; MFC = Minimum fungicidal concentration

Table 6: Effect of temperature on antifungal activity of clove oil (0.05 µL/mL)

Temperature (°C)	Mycelial growth (mm, 7 days)	Inhibition (%)	EO remaining in medium (% , after 7 days)
20	12.3 ± 1.2	86.3	68 ± 5
25	15.8 ± 1.5	82.4	55 ± 4
30	22.6 ± 2.1	74.9	42 ± 3
35	38.4 ± 3.2	57.3	28 ± 3
40	68.5 ± 5.1	23.9	12 ± 2

Data source: Sharma *et al.*, 2022

Table 7: Comparison of contact and vapor phase activity of selected EOs against *A. solani*

EO	Concentration (µL/plate)	Inhibition - Contact (%)	Inhibition - Vapor (%)
Thyme	10	86	52
Thyme	50	98	89
Clove	10	82	48
Clove	50	96	85
Lemongrass	10	71	42
Lemongrass	50	92	78

Data from 90 mm plates, 7 days incubation

Table 8: Nanoencapsulation strategies for essential oils against *Alternaria* spp.

Nanocarrier	EO	Particle size (nm)	Encapsulation efficiency (%)	Efficacy improvement over free EO	Reference
Chitosan NP	Thyme	150-200	72-85	2.5× longer protection	Li <i>et al.</i> , 2023
Liposome	Clove	120-180	68-78	3.0× lower EC50	Garcia <i>et al.</i> , 2022
Solid lipid NP	Oregano	80-120	75-88	2.2× higher inhibition	Wang <i>et al.</i> , 2021
PLGA NP	Lemongrass	180-250	65-74	1.8× longer residual activity	Patel <i>et al.</i> , 2022
Nanoemulsion	Thyme + Clove	20-50	-	2.5× synergistic effect	Singh <i>et al.</i> , 2023
Cyclodextrin	Cinnamaldehyde	-	85-92	4.0× photostability	Chen <i>et al.</i> , 2022

Conclusion

This comprehensive review of the published literature (2010-2024) on the antifungal activity of essential oils against *Alternaria solani*, the causal agent of early blight in tomato, leads to the following conclusions:

- Phenolic-rich essential oils are highly effective *in vitro***
Thyme oil (*Thymus vulgaris*), clove oil (*Syzygium aromaticum*), and oregano oil (*Origanum vulgare*) consistently demonstrate the strongest mycelial growth inhibition, with EC50 values ranging from 0.02 to 0.04 µL/mL. At 0.10 µL/mL concentration, these oils achieve 95-99% inhibition, comparable to synthetic fungicides at recommended doses.
- The primary antifungal mechanism involves membrane disruption and multi-target effects**
Unlike single-target synthetic fungicides, EOs exert their action through membrane permeabilization, ROS generation, ergosterol biosynthesis inhibition, mitochondrial dysfunction, and suppression of cell wall degrading enzymes. This multi-site mechanism reduces the likelihood of resistance development.
- Significant variability exists in EO efficacy**
The activity depends on multiple factors including chemical composition (phenolic > aldehyde > terpene alcohol > terpene hydrocarbon), concentration (critical dose-dependent effect), fungal isolate (2–3-fold variation in sensitivity), temperature (reduced activity above 35°C), growth medium, and application method (contact more effective than vapor).
- Translation from lab to field remains challenging.**
Major obstacles include high volatility (2–5-day residual activity), phytotoxicity at effective concentrations (leaf damage at >0.10 µL/mL), higher cost compared to synthetic fungicides (3-10×), lack of standardization (quality variation), and limited field validation (only 15-20 field studies to date).
- Nanoencapsulation and synergistic combinations are the most promising solutions.** Chitosan nanoparticles, liposomes, and nanoemulsions can extend residual activity to 10-14 days, reduce phytotoxicity by 40-60%, and lower effective concentrations by 2-4-fold.

Synergistic blends (EO-EO or EO-fungicide) allow dose reduction while maintaining efficacy.

- Essential oils fit best in integrated disease management and organic production**
Rather than direct replacement of all synthetic fungicides, EOs are most valuable as rotational partners (to delay resistance), as post-harvest treatments (edible coatings), and in organic or low-input production systems where synthetic fungicides are restricted.

Final recommendation

Thyme oil, clove oil, and oregano oil, particularly in nanoencapsulated formulations at 0.05-0.08 µL/mL, are the most promising essential oils for development as botanical fungicides against *Alternaria solani*. However, before large-scale adoption, additional field validation, economic analysis, and regulatory approval are required. The growing consumer demand for organic produce and the continued spread of fungicide-resistant *A. solani* strains ensure that essential oils will play an increasingly important role in sustainable early blight management.

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