Bioefficacy of Essential Oils Against the Tomato Wilt Pathogen *Fusarium oxysporum* f. sp. *lycopersici*

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Abstract. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* represents a major constraint to tomato production worldwide. The escalating concerns regarding synthetic fungicides necessitate exploration of sustainable alternatives. This study evaluated the in vitro antifungal efficacy of seven essential oils against *F. oxysporum* f. sp. *lycopersici* strain 8115 using the poisoned food technique. Essential oils of orange, mint, clove, lemongrass, cinnamon bark, eucalyptus, and moringa were incorporated into potato dextrose agar at six concentrations ranging from 500 to 3000 mg/L using Tween 80 as an emulsifier. Mycelial growth inhibition was quantified by measuring radial colony diameter. Results revealed significant dose-dependent antifungal activity across all treatments, with marked variation in efficacy. Cinnamon bark oil demonstrated the highest potency, achieving near-complete inhibition at 3000 mg/L with substantial activity evident even at lower concentrations. Orange oil also exhibited strong inhibitory effects, attaining complete growth suppression at the highest dose. In contrast, moringa and mint oils displayed minimal efficacy across all tested concentrations. The ranking of antifungal potency was established as Cinnamon Bark Oil ≥ Orange Oil > Clove Oil ≈ Eucalyptus Oil > Lemongrass Oil > Mint Oil > Moringa Oil. These findings highlight the potential of cinnamon bark and orange essential oils for further investigation as botanical alternatives for management of tomato *Fusarium* wilt.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici*, essential oils, cinnamon bark oil, orange oil, antifungal activity, poisoned food technique, botanical fungicides

1 Introduction

Tomato (*Solanum lycopersicum* L.) stands as one of the most economically significant vegetable crops globally, valued for both fresh consumption and processing industries. However, tomato cultivation faces persistent challenges from various soilborne pathogens, among which *Fusarium oxysporum* f. sp. *lycopersici* emerges as a particularly devastating threat. This specialized forma specialis causes vascular wilt disease, characterized by rapid wilting, yellowing of lower leaves, and eventual plant death, leading to substantial yield losses that can reach epidemic proportions under favorable conditions [1, 2]. The pathogen's ability to persist in soil for extended periods as resistant chlamydospores, combined with its capacity to colonize the plant's vascular system, renders conventional management strategies largely ineffective [3].

Traditional disease management has heavily relied on synthetic chemical fungicides, which, despite their efficacy, present mounting environmental and health concerns. The indiscriminate application of these compounds has led to the development of fungicide-resistant pathogen strains, soil contamination, disruption of beneficial microbial communities, and accumulation of toxic residues in food products and ecosystems [4, 5]. Furthermore, increasing regulatory restrictions and growing consumer preference for organically produced food have intensified the demand for eco-friendly disease management alternatives. This scenario has prompted researchers to explore natural plant-based products as sustainable substitutes for synthetic fungicides.

Essential oils, secondary metabolites extracted from aromatic plants, have garnered considerable attention as potential biocontrol agents due to their broad-spectrum antimicrobial properties, low environmental persistence, and reduced likelihood of resistance development [6, 7]. These complex mixtures of volatile organic compounds possess multiple modes of action, including disruption of fungal cell membranes, interference with cellular respiration, inhibition of enzyme systems, and alteration of cellular homeostasis [8]. Among the diverse array of essential oils investigated for antifungal applications, several have

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demonstrated promising activity against *Fusarium* species.

Cinnamon essential oil, primarily composed of cinnamaldehyde, has exhibited potent antifungal properties against various plant pathogens. Studies have documented its ability to inhibit mycelial growth and spore germination through membrane disruption and interference with ergosterol biosynthesis. Similarly, clove oil, rich in eugenol, has shown significant antimicrobial activity attributed to its capacity to penetrate fungal cell walls and disrupt mitochondrial function [6]. Citrus-derived essential oils, particularly orange oil containing d-limonene as a major constituent, have demonstrated effectiveness against multiple fungal pathogens through their lipophilic nature, facilitating interaction with fungal membrane components. Lemongrass oil, characterized by high citral content, exhibits antifungal mechanisms involving membrane permeabilization and oxidative stress induction [9]. Eucalyptus oil, containing eucalyptol and other monoterpenes, has been reported to suppress Fusarium growth through multiple biochemical pathways [10]. Mint oils, despite variable composition, have shown moderate antifungal properties in some investigations. Moringa seed oil, while primarily valued for nutritional applications, has received limited attention regarding its antifungal potential.

Despite numerous individual studies on various essential oils, comparative evaluations of their efficacy against *F. oxysporum* f. sp. *lycopersici* under standardized conditions remain limited. Such comparative assessments are crucial for identifying the most promising candidates for further formulation development and field applications. The selection of appropriate screening concentrations is equally important, as it determines the practical applicability of these natural compounds in disease management scenarios [11].

Against this background, the present investigation was undertaken with the objective of evaluating and comparing the in vitro antifungal efficacy of seven commercially available essential oils—orange, mint, clove, lemongrass, cinnamon bark, eucalyptus, and moringa—against *F. oxysporum* f. sp. *lycopersici* using the poisoned food technique across a gradient of concentrations. This study aims to identify the most potent essential oil(s) that could serve as potential candidates for future development of botanical formulations for sustainable management of tomato *Fusarium* wilt.

2 Materials and Methods

2.1 Pathogens and Culture Maintainence

A virulent strain of *Fusarium oxysporum* f. sp. *lycopersici* (strain 8115) was obtained from the Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, India. The culture was maintained on potato dextrose agar (PDA) medium through regular sub-culturing at 28 ± 2°C and stored at 4°C for short-term preservation. Prior to each experiment, the culture was revived by transferring mycelial bits onto fresh PDA plates and incubating under standard conditions until adequate mycelial growth was obtained.

2.2 Essential Oils

Seven commercially available essential oils were procured from authenticated suppliers and used for the study (Table 1).

Table 1. List of essential oils tested against *Fusarium oxysporum* f. sp. *lycopersici*.

Essential Oil	Scientific Name
Orange Oil	*Citrus sinensis*
Mint Oil	*Mentha* spp.
Clove Oil	*Syzygium aromaticum*
Lemongrass Oil	*Cymbopogon citratus*
Cinnamon Bark Oil	*Cinnamomum verum*
Eucalyptus Oil	*Eucalyptus globulus*
Moringa Oil	*Moringa oleifera*

Selection of these oils was based on literature evidence of their antifungal properties and commercial availability. All oils were stored in amber-colored bottles at 4°C in dark conditions to prevent oxidation and photodegradation.

2.3 Preparation of Essential Oil Emulsions

Since essential oils are hydrophobic and immiscible with aqueous culture media, stock emulsions were prepared using Tween 80 (polyoxyethylene sorbitan monooleate) as a non-ionic emulsifying agent. Tween 80 facilitates the dispersion of essential oils in the culture medium without exhibiting significant antifungal activity at the concentrations used. Stock emulsions were prepared by thoroughly mixing the essential oil with Tween 80 (0.1%, v/v), under continuous stirring to ensure uniform dispersion of the oil droplets. These stock emulsions were then used to prepare various test concentrations by adding calculated volumes to molten PDA medium.

2.4 Poisoned Food Technique

The antifungal efficacy of essential oils was evaluated using the poisoned food technique, a widely adopted in vitro screening method. PDA medium was prepared,

sterilized by autoclaving at 121°C for 15 minutes, and cooled to approximately 45-50°C. Calculated volumes of essential oil stock emulsions were aseptically added to achieve final concentrations of 500, 1000, 1500, 2000, 2500, and 3000 mg/L. The medium was thoroughly mixed to ensure uniform distribution of the essential oil and immediately poured into sterile Petri plates (90 mm diameter). Control plates containing PDA medium supplemented with 0.1% (v/v) Tween 80 without essential oil were prepared simultaneously to assess fungal growth in the absence of essential oils.

After solidification of the medium, a 5 mm mycelial disc obtained from the actively growing margin of a 7-day-old *Fusarium oxysporum* f. sp. *lycopersici* culture was aseptically placed at the center of each plate. The inoculated plates were sealed with parafilm and incubated at 28 ± 2°C under ambient light conditions, which is a standard temperature for in vitro growth of *Fusarium oxysporum* f. sp. *lycopersici*. Experiments were conducted in a completely randomized design with three replications for each treatment concentration.

Radial mycelial growth was measured by recording the colony diameter in two perpendicular directions when the fungal growth in control plates reached the plate edges. The mean of the two measurements was calculated to represent the radial growth for each replicate.

The percentage of mycelial growth inhibition was calculated using the formula proposed by Vincent (1947):

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

where C represents the radial growth of the fungus in the control plate (mm), and T represents the radial growth of the fungus in the treatment plate (mm).

2.5 Statistical Analysis

All experiments were conducted in triplicate and data are presented as mean ± standard deviation (SD). The experimental data were analyzed using two-way analysis of variance (ANOVA) to determine the effects of essential oil type, concentration, and their interaction on mycelial growth inhibition. Mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test at $p \leq 0.05$. (Table 2).

3 Results and Discussion

The in vitro evaluation of seven essential oils against *F. oxysporum* f. sp. *lycopersici* revealed substantial variation in antifungal efficacy, with all treatments exhibiting clear dose-dependent inhibition patterns. This concentration-response relationship confirms genuine antifungal activity rather than random effects, supporting the therapeutic potential of these natural compounds.

3.1 Comparative Efficacy of Essential Oils

The poisoned-food technique successfully demonstrated differential antifungal activity among the seven essential oils tested against the *F. oxysporum* f. sp. *lycopersici* strain 8115. Across the concentration gradient of 500 to 3000 mg/L, all treatments exhibited measurable inhibitory effects, though with markedly divergent potencies. Visual assessment of mycelial growth on PDA plates revealed distinct patterns of growth suppression, ranging from near-complete inhibition to minimal efficacy, enabling clear differentiation of treatment performance (Fig. 1). Among the tested essential oils, cinnamon bark oil emerged as the most potent antifungal agent, achieving approximately 100% growth inhibition at 3000 mg/L and exhibiting substantial activity even at lower concentrations (approximately 96% inhibition at 1500 mg/L). This exceptional efficacy can be attributed to cinnamaldehyde, the predominant bioactive constituent, which comprises 65-85% of the composition of cinnamon bark oil. Cinnamaldehyde exerts multifaceted antifungal effects by disrupting fungal cell membrane integrity through interference with ergosterol biosynthesis, inhibiting cell wall formation, perturbing amino acid decarboxylase activity, and inducing oxidative stress via reactive oxygen species generation [6, 12]. Orange oil demonstrated the second-highest antifungal efficacy, achieving complete growth inhibition at 3000 mg/L and approximately 95% inhibition at 2500 mg/L. The antimicrobial properties of orange oil are primarily attributed to d-limonene, a monoterpene hydrocarbon constituting 90-95% of the essential oil. The mechanism of action involves membrane disruption through lipid peroxidation, leading to increased membrane permeability, leakage of intracellular contents, and eventual cell death. Additionally, limonene interferes with mitochondrial function and induces morphological alterations in fungal hyphae, including irregular branching and hyphal distortion [8]. The strong performance of orange oil positions it as a cost-effective alternative to cinnamon bark oil, particularly considering its widespread commercial availability and relatively lower market price.

The remaining essential oils—clove, eucalyptus, and lemongrass—exhibited moderate antifungal activity, achieving inhibition levels ranging from 55% to 64% at 3000 mg/L. Clove oil (approximately 64% inhibition), rich in eugenol, demonstrated membrane-disrupting properties through interference with ergosterol synthesis and cytoplasmic membrane integrity [6]. Eucalyptus oil (approximately 63% inhibition) exerted its effects primarily through 1,8-cineole (eucalyptol) and α-pinene, which disrupt membrane structure and interfere with respiratory enzymes [10]. Lemongrass oil (approximately 55% inhibition) showed activity attributable to citral content, which induces membrane permeabilization and inhibits ergosterol biosynthesis [9]. While these oils demonstrated statistically significant inhibitory effects, their moderate potency suggests limited standalone utility for field applications

without formulation optimization or synergistic combinations.

Mint oil and moringa oil displayed the weakest antifungal activity among all tested treatments, achieving only 47% and 28% inhibition respectively at the maximum concentration of 3000 mg/L. The limited efficacy of mint oil may reflect compositional variations influenced by species differences, geographic origin, and extraction methodology, as menthol-menthone ratios significantly affect antimicrobial potency [13]. Moringa oil exhibited minimal activity across all concentrations, likely due to its distinct chemical composition dominated by oleic acid and other fatty acids rather than volatile terpenes and phenolic compounds characteristic of typical essential oils with strong antimicrobial properties.

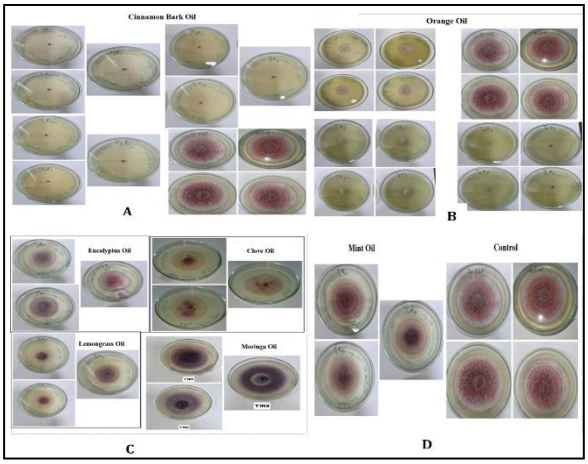

Fig. 1. Representative culture plates illustrating the antifungal activity of different essential oils against *Fusarium oxysporum* f. sp. *lycopersici* using the poisoned food technique. (A) Cinnamon bark oil showing maximum inhibition of mycelial growth, (B) Orange oil exhibiting strong antifungal activity with marked reduction in fungal growth, (C) Eucalyptus, clove, lemongrass, and moringa oils showing moderate inhibitory effects, and (D) Mint oil and untreated control plates showing comparatively higher fungal growth. Progressive inhibition of mycelial development was observed depending on the concentration of essential oil applied.

3.2 Integrated Visualization of Antifungal Efficacy Patterns

To provide a comprehensive visualization of the differential antifungal efficacy across all treatments and concentrations, the inhibition data were represented as a heatmap (Fig. 2). This visualization enables rapid identification of efficacy patterns and facilitates comparative assessment of treatment performance. The color gradient intensity directly corresponds to inhibition levels, with darker red tones indicating higher percent inhibition (>90%) and lighter yellow tones representing lower inhibition (<30%). The heatmap clearly delineates three distinct categories of essential oil performance: (i) highly potent oils (cinnamon bark and orange) exhibiting rapid transition to maximum inhibition across the concentration gradient, (ii) moderately effective oils (clove, eucalyptus, and lemongrass) showing gradual increase in inhibition with concentration, and (iii) weak inhibitors (mint and moringa) remaining predominantly in the low inhibition zones even at maximum tested concentrations. This visual representation underscores the superior dose-response relationship of cinnamon bark oil, which achieved near-complete inhibition at concentrations as low as 1500 mg/L, in stark contrast to moringa oil which exhibited minimal activity across the entire concentration range.

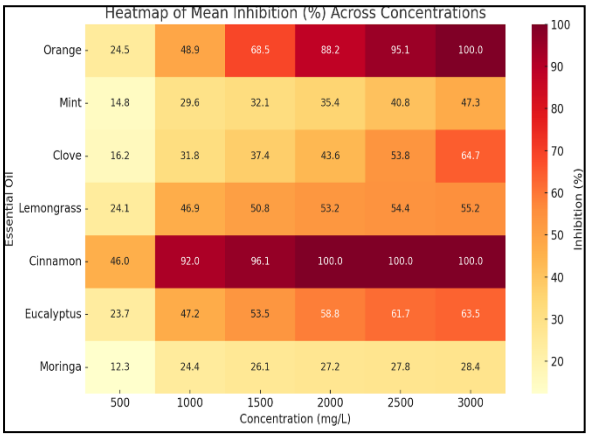

Fig. 2. Heatmap visualization depicting the concentration-dependent antifungal efficacy of seven essential oils against *Fusarium oxysporum* f. sp. *lycopersici*. Color intensity represents percent mycelial growth inhibition: dark red (90-100% inhibition), orange-red (60-90% inhibition), light orange (30-60% inhibition), and yellow (<30% inhibition). Each cell represents mean inhibition values from three independent replicates. The heatmap facilitates rapid comparative assessment of treatment efficacy, clearly distinguishing highly potent oils (cinnamon bark, orange) from weak inhibitors (mint, moringa).

3.3 Comparative Statistical Analysis of Essential Oil Treatments

Two-way analysis of variance (ANOVA) indicated that essential oil treatment, concentration, and their interaction significantly affected the mycelial growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* ($p \leq 0.05$). Among all treatments, cinnamon bark oil exhibited significantly greater antifungal activity than the other essential oils across the tested concentration range, achieving complete inhibition at concentrations of 2000 mg/L and above. Orange oil demonstrated the second-highest inhibitory activity and also produced complete growth inhibition at the highest concentration tested. In contrast, moringa oil consistently exhibited the lowest inhibition values throughout the experiment. The significant interaction between treatment and concentration indicated that the response of *F. oxysporum* f. sp. *lycopersici* to increasing concentrations differed among the evaluated essential oils. Tukey's HSD multiple comparison test further confirmed the superiority of cinnamon bark oil and

orange oil, which formed the highest statistical group ('a') and differed significantly from the remaining treatments. Clove oil and eucalyptus oil constituted an intermediate group, whereas mint oil and moringa oil exhibited significantly lower antifungal activity.

Table 2. Mean mycelial growth inhibition (%) of different essential oils against *Fusarium oxysporum* f. sp. *lycopersici* at 3000 mg/L. Means followed by different letters differ significantly according to Tukey's HSD test ($p \leq 0.05$).

Essential Oil	Mean Inhibition % with Tukey Group
Cinnamon Bark Oil	100.0[a]
Orange Oil	100.0[a]
Clove Oil	64.7[b]
Eucalyptus Oil	63.5[b]
Lemongrass Oil	55.2[bc]
Mint Oil	47.3[c]
Moringa Oil	28.4[d]

Tukey's HSD multiple comparison test further confirmed the superiority of cinnamon bark oil and orange oil, which formed the highest statistical group ('a') and differed significantly from the remaining treatments. Clove oil and eucalyptus oil constituted an intermediate group, whereas mint oil and moringa oil exhibited significantly lower antifungal activity. These results indicate that cinnamon bark oil and orange oil were the most effective treatments against *Fusarium oxysporum* f. sp. *lycopersici* under the tested conditions.

3.4 Mechanisms of Antifungal Action and Future Prospects

The antifungal mechanisms of essential oils are complex and multifaceted, involving interactions with multiple cellular targets. The primary mode of action involves disruption of fungal cell membrane integrity. Essential oil constituents, being predominantly lipophilic compounds, readily partition into the lipid bilayer of fungal cell membranes, causing structural perturbations, increased membrane permeability, and leakage of intracellular components [6, 8].

Secondary mechanisms include interference with ergosterol biosynthesis, a crucial component of fungal cell membranes. Essential oil constituents inhibit enzymes involved in the ergosterol biosynthetic pathway, particularly squalene epoxidase and C-14 demethylase. Additionally, essential oils induce oxidative stress through generation of reactive oxygen species, overwhelming cellular antioxidant defenses and causing damage to lipids, proteins, and nucleic acids.

Essential oils also interfere with fungal energy metabolism by disrupting mitochondrial function and inhibiting respiratory enzymes, leading to reduced ATP production. Furthermore, these compounds affect fungal morphogenesis and development by inhibiting spore germination, reducing germ tube elongation, causing hyphal distortion, and suppressing sporulation capacity. The multi-target nature of essential oil action reduces the likelihood of resistance development compared to single-site synthetic fungicides, representing a significant advantage for sustainable disease management strategies [14, 7].

Based on the comprehensive evaluation, the ranking of essential oils by antifungal potency against *F. oxysporum* f. sp. *lycopersici* was established as: Cinnamon Bark Oil ≥ Orange Oil > Clove Oil ≈ Eucalyptus Oil > Lemongrass Oil > Mint Oil > Moringa Oil.

Cinnamon bark oil and orange oil emerge as the most promising candidates warranting advancement to subsequent research phases, including determination of precise effective concentration values (EC_{50}, MIC), development of stable emulsion formulations, evaluation of synergistic combinations with other biocontrol agents, assessment of phytotoxicity on tomato plants, investigation of formulation stability and shelf life, and validation under greenhouse and field conditions [15, 16].

The integration of these essential oils into integrated disease management programs may offer potential for reducing dependence on synthetic fungicides; however, further greenhouse and field validation studies are required. Previous investigations have explored various application strategies including seed treatment, soil drenching, foliar sprays, and incorporation into controlled-release formulations. Furthermore, combination approaches integrating essential oils with biological control agents such as *Bacillus* spp. or *Trichoderma* spp. have shown enhanced disease suppression [14].

While in vitro screening provides valuable preliminary data, several limitations must be acknowledged. The in vitro conditions differ substantially from complex soil-plant-pathogen interactions occurring in natural systems. Therefore, while cinnamon bark and orange oils demonstrated exceptional in vitro activity, their performance under field conditions requires rigorous validation. Additionally, practical considerations including cost-effectiveness, availability, environmental impact, and regulatory approval processes must be evaluated before commercial deployment.

4 Conclusion

This comparative in vitro evaluation has identified cinnamon bark oil and orange oil as highly potent antifungal agents against *Fusarium oxysporum* f. sp. *lycopersici,* demonstrating near-complete growth

inhibition at 3000 mg/L with substantial activity at lower concentrations. The clear dose-dependent response observed across all tested essential oils validates their genuine antifungal properties, while the marked variation in efficacy underscores the importance of systematic screening under standardized conditions. Lemongrass, clove, and eucalyptus oils exhibited moderate activity, whereas mint and moringa oils showed limited practical utility.

These findings contribute to the growing body of evidence supporting the potential of plant-derived essential oils as sustainable alternatives or complements to synthetic fungicides in management of soilborne plant diseases. The multi-target mode of action characteristic of essential oils reduces the risk of resistance development, while their natural origin aligns with increasing demand for environmentally benign crop protection strategies. However, translation from in vitro efficacy to field application requires addressing several challenges, including formulation optimization, determination of economically viable application rates, assessment of potential phytotoxicity, and integration into holistic disease management programs.

Future research should focus on precise quantification of effective concentration parameters (EC_{50}, minimum inhibitory concentration) for the most promising oils, chemical profiling to identify key bioactive constituents, investigation of synergistic combinations with other essential oils or biocontrol agents, development of nano-emulsion or encapsulation technologies, evaluation of formulation performance in greenhouse and field experiments, comparison with commercial synthetic fungicides, assessment of impacts on soil microbial communities and plant growth promotion, and economic analysis of production and application costs. Through such comprehensive investigations, botanical essential oils could emerge as valuable tools in sustainable integrated management of tomato *Fusarium* wilt, contributing to reduced chemical fungicide dependency while maintaining productive and profitable tomato cultivation systems.

Conflict of Interest: All authors declare no conflict of interest.

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