Essential Oils as Potential Antimicrobial Agents for Biological Threats: Focus on Thymol-Based Green Disinfectant

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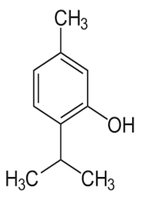
Abstract

Biological agents are hazardous and necessitate effective disinfectants for the well-being and security of the general public. We examined the antibacterial properties of four Essential Oils (EOs) by Minimum Inhibitory Concentration (MIC) evaluation. Thymol displayed the most effective antimicrobial activity, Carvacrol, Eugenol, and Menthol followed in descending sequence. Minimum Bactericidal Concentration (MBC) was also assessed. Moreover the antimicrobial activity of EOs was investigated through dose-response and time course experiments. The activity was fully manifested after one minute of incubation. Thymol, is a promising innovative antimicrobial agents possessing potential applications in contexts involving microbial threats and addressing the growing demand for natural disinfectants.

Overview

EOs are volatile secondary metabolites produced by plants that contribute to a distinctive aroma or flavor. Since ancient times, EOs have been utilized for various purposes. Their biological properties have also made them valuable in medical applications. Several EOs, such as Eugenol, Carvacrol, Thymol, and Menthol, possess antimicrobial properties, which are highly significant in scientific and industrial fields. Thymol, a natural monoterpenoid phenol and isomer of Carvacrol, is abundant in EOs derived from plants of the Thymus genus. It exerts its antimicrobial effects primarily by damaging cell membranes, disrupting the proton motive force and energy production, harming cellular homeostasis, and releasing intracellular material, including ATP.



MIC and MBC evaluation of Essential Oils

To assess the antimicrobial activity of Carvacrol, Eugenol, Menthol, and Thymol, we determined MIC for each EO against two reference strains, *Staphylococcus aureus* ATCC 6538 (Gram positive) and *Escherichia coli* ATCC 8739 (Gram negative). The MIC is the lowest concentration of an antimicrobial agent that, under specific in vitro conditions, prevents visible growth within a defined time. Our results showed that Thymol had the lowest MIC, which was at least eight times lower than the other tested compounds, indicating superior antimicrobial activity compared to the other EOs. Carvacrol, Eugenol, and Menthol followed in descending order of effectiveness, with Menthol having the highest MIC. We also evaluated the MBC, which represents the minimum concentration of the antibacterial agent that reduces bacterial viability by 99.9% or more. For the tested EOs, the MBC was higher than the MIC, suggesting that a slightly higher concentration is required for complete bacterial killing.

Essential Oils	*S. aureus*		*E. coli*		Ranking of EOs AM activity
	MIC (%)	MBC (%)	MIC (%)	MBC (%)	
Thymol	1.5×10^{-5}	2.5×10^{-4}	0.005	0.005	1
Carvacrol	0.04	0.08	0.04	0.04	2
Eugenol	0.04	0.15	0.08	0.31	3
Menthol	3.9	N.O.	3.9	N.O.	4

Table 1. Table of the antimicrobial activity of four different EOs.
N.O.: not observed; AM: antimicrobial.

Figure 1. MIC (red circle) experiment on Thymol against *S. aureus* and *E. coli*.

Summary & outlook

The significance of this research lies in the potential application of EOs based disinfectants in microbial treaths contexts, emphasizes addressing the critical need for reliable and eco-friendly solutions. The study focuses mainly on the role of Thymol as a promising candidate for the development of innovative and sustainable disinfectants to mitigate the impact of hazardous incidents on public health and safety.

Results and future prospects

Thymol's antimicrobial activity was investigated also by dose-response and time course experiments, providing a comprehensive understanding of how the molecule concentration and exposure time affects bacterial growth. Following these assessments, the disinfectant will undergo additional rigorous testing on major bioterrorism agents to ensure its efficacy against a wide range of agents.

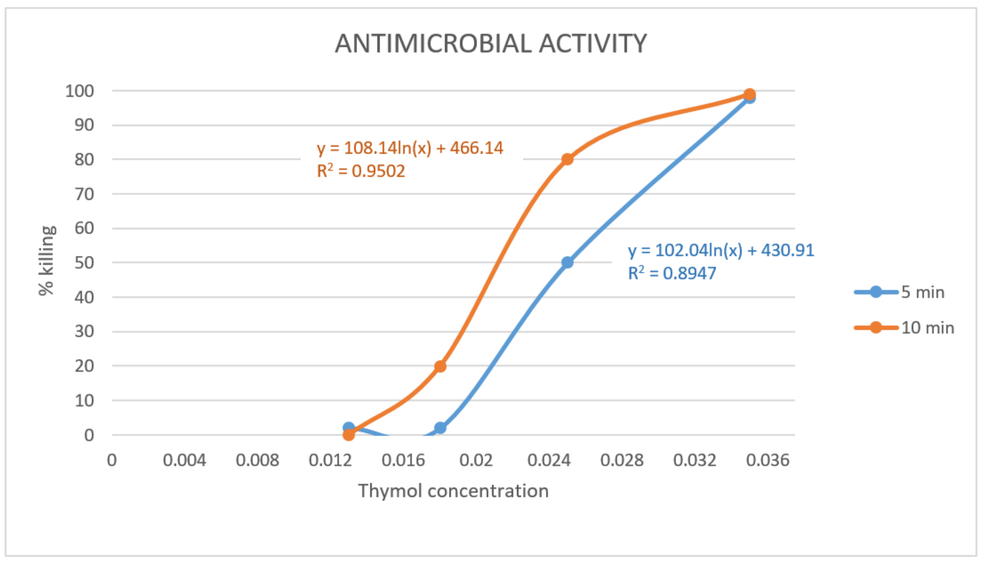


Figure 2. Thymol's killing rate on *S. aureus*.

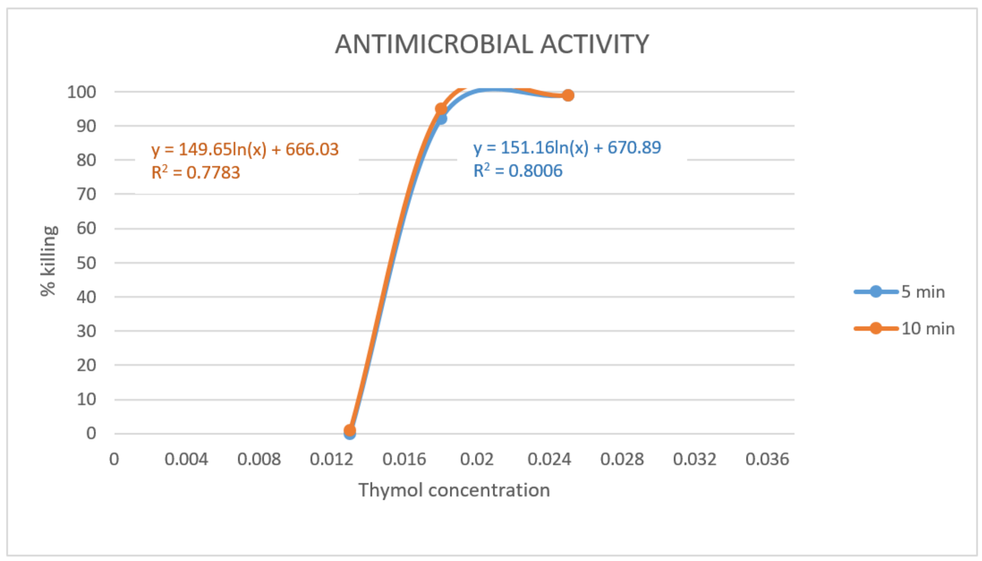


Figure 3. Thymol's killing rate on *E. coli*.

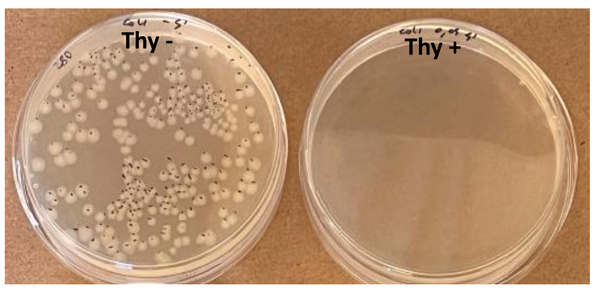


Figure 4. Thymol's inibition on *E. coli*.
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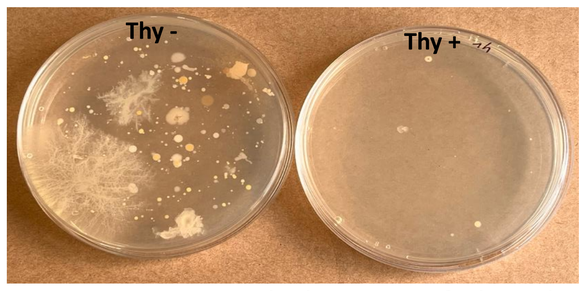

Figure 5. Antimicrobial activity of Thymol (0.05%) on sample simulating environmental specimen (1h of incubation).

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