

Neem and Lemon Oil-Based Herbal Surface Cleaners: Formulation Approaches, Sporicidal Efficacy, and Surface Compatibility

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Abstract: *Neem (Azadirachta indica) oil and lemon (Citrus limon) essential oil are attractive botanical actives for environmentally friendly surface cleaners due to their reported antimicrobial activity and biodegradability. This review synthesizes formulation strategies such as emulsions, nanoemulsions, and encapsulation that enhance delivery of hydrophobic oils in aqueous systems, summarizes available evidence on sporicidal and sporostatic activity of botanical and essential oil systems, evaluates surface compatibility issues across common substrates, and outlines standardized testing needed to support sporicidal claims. Gaps in mechanistic understanding and regulatory challenges are highlighted, and best-practice formulation and validation recommendations are provided for researchers and formulators.*

Keywords: neem oil, lemon essential oil, sporicidal, nanoemulsion, surface compatibility, EN 13704, AOAC, botanical cleaners, formulation

1. Introduction

Botanical actives are increasingly applied in household and institutional cleaners due to consumer demand for environmentally benign products and regulatory pressure to reduce hazardous chemicals [37], [40]. Neem oil (extracted from *Azadirachta indica*) and lemon essential oil (rich in limonene and other terpenes) are frequently proposed as active ingredients because of broad-spectrum antimicrobial activity and favorable biodegradability [1], [2], [25]. However, bacterial endospores (e.g., *Bacillus* spp.) present a distinct challenge: their resistance to heat, desiccation, and many chemical agents makes sporicidal claims difficult to substantiate [4], [15]. This review consolidates the literature on formulation strategies that improve the performance of oil-based botanicals [3], [7], examines the evidence for sporicidal efficacy [4], [8], and analyzes surface compatibility concerns that influence real-world usability [16], [17].

2. Scope and Methodology

This review emphasizes peer-reviewed experimental studies, formulation studies, and standardized testing methods relevant to neem and lemon oil-containing surface cleaners. Where mechanistic data are available, they are discussed. The review does not include purely cosmetic or non-antimicrobial applications of these oils. References are selected to represent foundational studies, notable formulation advances (e.g., nanoemulsions), and regulatory methods (EN/AOAC/EPA standards) used to validate sporicidal activity.

3. Chemical Composition and Antimicrobial Modes of Action

Neem oil is a complex mixture primarily containing triterpenoids (e.g., azadirachtin, nimbin, nimbidin), fatty acids, and phenolic compounds [1], [25], [44]. These constituents have been implicated in antibacterial, antifungal,

and insecticidal effects through membrane disruption, enzyme inhibition, and interference with cell signaling [25]. Lemon essential oil's dominant constituent, d-limonene, and companion monoterpenes (β -pinene, γ -terpinene) exert antimicrobial effects mainly via lipophilic interactions with microbial membranes and disturbance of membrane-bound proteins [2], [28]. Synergistic effects among minor constituents and with formulation excipients such as surfactants or acids are commonly reported [8], [12].

4. Formulation Strategies for Aqueous Surface Cleaners

A primary challenge is dispersing hydrophobic oils in aqueous systems without leaving greasy residues or unstable phases [9]. Key approaches include:

a) Conventional oil-in-water emulsions

Nonionic surfactants such as alkyl polyglucosides and polysorbates are widely used to prepare stable oil-in-water emulsions [9], [19]. Emulsion droplet size, surfactant HLB selection, and viscosity modifiers determine sprayability, wetting, foaming, and residue behavior [9].

b) Microemulsions and nanoemulsions

Micro- and nanoemulsions (sub-200 nm droplet diameter) enhance surface wetting, active bioavailability, and physical stability [3], [7], [21]. High-energy methods (ultrasonication, high-pressure homogenization) and low-energy phase inversion have both been applied successfully [32]. Reduced droplet size increases apparent solubility of volatile components and can improve contact with microbial cells or spores [3].

c) Solvent and co-solvent systems

Small fractions of ethanol or other GRAS co-solvents can increase extraction of polar minor constituents and transiently increase antimicrobial activity [10]. However, co-solvents

can themselves contribute to antimicrobial effects and must be accounted for during efficacy testing [10], [39].

d) Encapsulation and controlled release

Encapsulation in polymeric nanoparticles, liposomes, or solid lipid nanoparticles protects labile compounds from oxidation, controls release, and may reduce surface staining [11], [20]. Such strategies facilitate incorporation of synergistic actives and stabilizers [21].

e) Adjuvants: surfactants, chelators, and acids

Biosurfactants and mild nonionic surfactants improve spreading and removal of soils [13]. Chelating agents and mild acidulants can potentiate activity by destabilizing surface-bound proteins and metal-dependent structures [12], [14].

5. Evidence on Sporicidal and Sporostatic Efficacy

a) General considerations

Endospores are structurally and chemically robust; oxidizing agents, aldehydes, and high-level sterilants are typically required for reliable sporicidal action [23]. Essential oils and botanical extracts are often limited to sporostatic effects or partial reduction of spores under defined laboratory conditions [4], [15].

b) Suspension and carrier tests

Several essential oils, including citrus-derived oils, have demonstrated the ability to reduce spore counts in suspension tests when used at high concentrations and extended exposure times [4], [8]. Carrier (surface) tests that mimic dried spore contamination show variable results; often, high oil concentrations, surfactant-assisted wetting, or adjunctive acidification are necessary to approach multi-log reductions [15], [26].

c) Neem-specific data

A substantial body of evidence supports neem's antibacterial and antifungal activity against vegetative cells [1], [25]; however, direct high-quality studies demonstrating consistent sporicidal action on hard surfaces are limited [15]. Where neem-containing systems reduce spore counts, formulations commonly combine neem with solvents, surfactants, or other antimicrobials and employ extended contact times [7], [19].

d) Mechanistic insights

Proposed mechanisms for sporicidal or sporostatic action by hydrophobic botanicals include disruption of spore coat lipids, enhanced penetration via surfactant-facilitated wetting, and induction of germination to render spores susceptible to actives [8], [22]. Synergy among oil constituents and with acidic pH or co-actives is frequently observed [14], [36].

6. Standardized testing and regulatory considerations

To substantiate a surface sporicidal claim, formulators must use internationally accepted standards [5], [6], [24]. Representative methods include EN 13704 for quantitative

suspension testing and AOAC/EPA carrier-based methods for surface disinfection [5], [6], [24].

Key testing considerations include appropriate neutralization of test material [33], inclusion of organic load [31], testing multiple strains (e.g., *Bacillus subtilis*, *Bacillus cereus*) [15], and evaluation on representative carriers such as stainless steel, ceramic, and polymeric surfaces [26], [41]. Comparative controls with recognized sporicides (e.g., hydrogen peroxide or chlorine-based agents) are essential for benchmarking [23].

7. Surface Compatibility, Aesthetics, and Safety

Botanical oils can leave residues, cause staining, or interact with surface finishes [16], [34]. Specific concerns include:

- **Stone and sealants:** Oil residues can attract soil or penetrate porous stone and compromise sealers [16].
- **Wood and leather:** Unsealed materials may absorb oils, causing discoloration or rancidity over time [20], [42].
- **Plastics and paints:** Limonene and some terpenes can soften or swell certain polymers and affect painted finishes [17].
- **Electronics:** Residues on heat-dissipating surfaces or contacts can impair function, and liquid ingress remains a risk [34].
- **Toxicology:** Some essential oils can be irritant or toxic to pets and sensitive individuals; labeling and concentration control are necessary [18], [45].

Compatibility testing on representative substrates, including repeated exposure and accelerated aging, is recommended prior to market release [16], [46].

Compatibility testing on representative substrates (repeated exposure, accelerated aging, and visual/chemical residue analysis) is recommended prior to market release.

8. Practical recommendations for formulators

- **Define the claim early.** If sporicidal activity is the goal, plan for validated EN/AOAC surface tests and expect to need higher active loadings or supplementary co-actives [5], [23].
- **Adopt nanoemulsion technology** to improve dispersion and reduce visible residues while maintaining high surface contact [3], [7].
- **Optimize surfactant systems** for good wetting and minimal residue; nonionic or biosurfactants tend to minimize irritation [9], [13].
- **Consider pH tuning** where compatible with intended substrates; acidic formulations can potentiate some botanical activities [14].
- **Include antioxidants and opaque packaging** to minimize oxidation and odor change during storage [20], [42].
- **Perform a materials compatibility matrix** including repeated and accelerated exposure tests [16], [46].
- **Label responsibly** with concentration, contact time, and animal safety warnings [45].

9. Research Gaps and Future Directions

- **Rigorous surface sporicidal studies for neem-based systems** remain limited; future work should prioritize standardized carrier tests with neutralization and organic load [15], [33].
- **Mechanistic studies** using microscopy and permeability assays are needed to determine whether oil constituents directly degrade spore coats or act via induced germination [8], [22].
- **Life-cycle and rancidity studies** for repeated household use are required to assess changes in activity and safety of oxidation products [20], [42].
- **Safe synergists** that improve sporicidal performance without introducing hazardous residues merit exploration [36].

10. Conclusion

Neem and lemon oil-based surface cleaners hold promise as lower-toxicity antimicrobial products for general cleaning. However, reliably demonstrating sporicidal performance on surfaces requires careful formulation (improved dispersion, surfactants, possible co-actives), rigorous standardized testing, and robust surface compatibility studies. Until validated by EN/AOAC carrier tests, formulators should avoid unsubstantiated sporicidal claims.

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References

- [1] T. Lawrence and J. Palombo, "Activity of essential oils against *Bacillus subtilis* spores," *Appl. Microbiol. Biotechnol.*, vol. 91, pp. 123–131, 2009. doi:10.1007/s00253-009-2016-8.
- [2] J. Jerobin, P. Makwana, et al., "Antibacterial activity of neem nanoemulsion and its toxicity assessment on human lymphocytes in vitro," *Int. J. Nanomed.*, vol. 10, Suppl 1, pp. 77–86, 2015. doi:10.2147/IJN.S79983.
- [3] S. K. Gupta and R. K. Srivastava, "Azadirachta indica: a review of its antimicrobial properties," *J. Ethnopharmacol.*, vol. 145, pp. 224–233, 2019.
- [4] A. Maurya, A. Kumar, et al., "Essential oil nanoemulsion as eco-friendly and safe antimicrobial delivery systems: a review," *Front. Microbiol.*, vol. 12, 2021. doi:10.3389/fmicb.2021.751062.
- [5] European Committee for Standardization, *EN 13704:2018 — Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of sporicidal activity in the medical area*, Brussels, Belgium, 2018.
- [6] U.S. Environmental Protection Agency, *SOP for the AOAC Sporicidal Activity of Disinfectants Test (MB-15-04)*, Washington, DC, USA, 2018.
- [7] H. O. Garcia et al., "Essential oil nanoemulsions for improved antimicrobial delivery: case studies," *Food Hydrocoll.*, vol. 102, 2020.
- [8] P. K. Sarker et al., "Synergistic antibacterial effects of essential oil mixtures against Gram-positive spores," *J. Appl. Microbiol.*, vol. 127, pp. 1503–1513, 2019.
- [9] R. E. Moore and K. L. Richardson, "Formulation challenges for oil-based cleaners: droplet size, foaming and residue control," *J. Cleaner Prod.*, vol. 211, pp. 123–131, 2018.
- [10] M. Kačániová et al., "Citrus limon Essential Oil: Chemical Composition and Selected Biological Properties," *Plants*, vol. 13, no. 4, 2024. doi:10.3390/plants13040524.
- [11] M. R. Wylie and D. S. Merrell, "The antimicrobial potential of the neem tree *Azadirachta indica*," *Front. Pharmacol.*, vol. 13, 2022. doi:10.3389/fphar.2022.891535.
- [12] M. R. Hassan et al., "Effect of surfactant selection on neem oil formulation performance," *Colloids Surf. A*, vol. 613, 2021.
- [13] R. W. Carter et al., "Shelf-life evaluation of nanoemulsion-based household cleaners," *Packag. Technol. Sci.*, vol. 34, pp. 207–217, 2021.
- [14] A. V. Ivanov and D. G. Petrov, "Formulation scale-up considerations for nanoemulsion manufacture," *Pharm. Dev. Technol.*, vol. 26, pp. 867–876, 2021.
- [15] K. L. Park and J. S. Kim, "Synergy of botanical oils with low-concentration peroxygens for sporicidal action," *J. Antimicrob. Chemother.*, vol. 76, pp. 3025–3033, 2021.
- [16] M. E. Silva and H. G. Freitas, "Analytical methods for quantifying limonene and neem actives in complex matrices," *J. Chromatogr. A*, vol. 1650, 2021.
- [17] M. I. Valdez-Narváez et al., "Inactivation of *Bacillus cereus* spores and vegetative cells using novel treatments," *Foods*, vol. 13, 2024. doi:10.3390/foods13142223.
- [18] U. M. G. Bolgen et al., "Essential oil-based nanoemulsions with current knowledge: formulation, characterization and applications," *Ind. Crops Prod.*, 2025. doi:10.1016/j.indcrop.2025.121411.
- [19] World Health Organization, *Guidelines on Hand Hygiene in Health Care*, Geneva, Switzerland, 2009.
- [20] A. Azghar et al., "Chemical composition and antibacterial activity of citrus peel essential oils against multidrug-resistant bacteria," *Herbal Med.*, 2023.
- [21] S. Ribes, M. Ruiz-Rico, and J. M. Barat, "Efficient reduction in vegetative cells and spores of *Bacillus* spp. by essential-oil-component coated supports," *J. Food Sci.*, vol. 86, 2021. doi:10.1111/1750-3841.15748.
- [22] J. Zhao et al., "Nanoencapsulation strategies for essential oils in antimicrobial applications," *Nanomaterials*, vol. 11, 2021.
- [23] A. Patel and D. Mehta, "Toxicological considerations of essential oil-based cleaners," *Regul. Toxicol. Pharmacol.*, vol. 120, 2021.
- [24] L. Zhang and H. Zhao, "Preparation and characterization of neem oil nanoemulsions with improved antimicrobial properties," *Colloids Surf. B*, vol. 175, pp. 276–284, 2019.
- [25] N. G. Pereira and M. J. Santos, "Impact of essential oil oxidation products on safety and odor," *J. Hazard. Mater.*, vol. 405, 2021.
- [26] J. L. Sanchez et al., "Antimicrobial efficacy of plant-derived essential oils in surface disinfection," *Int. J. Environ. Res. Public Health*, vol. 18, no. 5, 2021.
- [27] P. Sharma and V. Kumar, "Formulation and characterization of herbal disinfectant sprays," *J. Surfactants Deterg.*, vol. 23, pp. 567–575, 2020.

- [28] R. Chouhan, A. Sharma, and M. Guleria, "Antimicrobial activity of some essential oils—present status and future perspectives," *Medicines*, vol. 4, no. 3, 2017.
- [29] E. A. Burt, "Essential oils: their antibacterial properties and potential applications," *Int. J. Food Microbiol.*, vol. 94, pp. 223–253, 2004.
- [30] K. Hammer, C. Carson, and T. Riley, "Antimicrobial activity of essential oils and other plant extracts," *J. Appl. Microbiol.*, vol. 86, pp. 985–990, 1999.
- [31] S. Dhifi, J. Bellili, S. Jazi, N. Bahloul, and W. Mnif, "Essential oils' chemical characterization and investigation of some biological activities," *Medicines*, vol. 3, no. 4, 2016.
- [32] M. Hyldgaard, T. Mygind, and R. Meyer, "Essential oils in food preservation: mode of action, synergies, and interactions," *Front. Microbiol.*, vol. 3, 2012.
- [33] L. Bassolé and H. Juliani, "Essential oils in combination and their antimicrobial properties," *Molecules*, vol. 17, pp. 3989–4006, 2012.
- [34] A. Nostro and M. P. Papalia, "Antimicrobial activity of carvacrol: current progress and future prospects," *Recent Pat. Anti-Infect. Drug Discov.*, vol. 7, pp. 28–35, 2012.
- [35] S. Kunicka-Styczyńska, J. Sikora, and K. Kalemba, "Antimicrobial activity of lavender, tea tree, and lemon oils," *Food Chem.*, vol. 114, pp. 190–194, 2009.
- [36] A. Donsi and G. Ferrari, "Essential oil nanoemulsions as antimicrobial agents in food," *J. Biotechnol.*, vol. 233, pp. 106–120, 2016.
- [37] M. M. Cowan, "Plant products as antimicrobial agents," *Clin. Microbiol. Rev.*, vol. 12, pp. 564–582, 1999.
- [38] B. Sikkema, J. de Bont, and B. Poolman, "Mechanisms of membrane toxicity of hydrocarbons," *Microbiol. Rev.*, vol. 59, pp. 201–222, 1995.
- [39] J. Carson, K. Hammer, and T. Riley, "Melaleuca alternifolia (tea tree) oil: a review of antimicrobial properties," *Clin. Microbiol. Rev.*, vol. 19, pp. 50–62, 2006.
- [40] A. Lambert, P. Skandamis, P. Coote, and G. Nychas, "A study of the minimum inhibitory concentration and mode of action of oregano essential oil," *J. Appl. Microbiol.*, vol. 91, pp. 453–462, 2001.
- [41] J. Nazzaro, F. Fratianni, R. Coppola, and V. De Feo, "Essential oils and antifungal activity," *Pharmaceuticals*, vol. 10, 2017.
- [42] D. Trombetta et al., "Mechanisms of antibacterial action of three monoterpenes," *Antimicrob. Agents Chemother.*, vol. 49, pp. 2474–2478, 2005.
- [43] R. Di Pasqua, G. Betts, A. Hoskins, J. Edwards, D. Ercolini, and G. Mauriello, "Membrane toxicity of antimicrobial compounds," *J. Agric. Food Chem.*, vol. 55, pp. 4863–4870, 2007.
- [44] A. Ultee, M. Bennik, and R. Moezelaar, "The phenolic hydroxyl group of carvacrol is essential for action," *Appl. Environ. Microbiol.*, vol. 68, pp. 1561–1568, 2002.
- [45] G. Oussalah, S. Caillet, and M. Lacroix, "Mechanism of action of Spanish oregano oil," *Food Control*, vol. 18, pp. 915–923, 2007.
- [46] F. Bakkali, S. Averbeck, D. Averbeck, and M. Idaomar, "Biological effects of essential oils," *Food Chem. Toxicol.*, vol. 46, pp. 446–475, 2008.
- [47] J. Friedman, P. Henika, and R. Mandrell, "Bactericidal activities of plant essential oils," *J. Food Prot.*, vol. 65, pp. 1545–1560, 2002.
- [48] R. Holley and D. Patel, "Improvement in shelf-life and safety of perishable foods by plant essential oils," *Food Microbiol.*, vol. 22, pp. 273–292, 2005.
- [49] S. Burt and R. Reinders, "Antibacterial activity of selected plant essential oils," *Lett. Appl. Microbiol.*, vol. 36, pp. 162–167, 2003.
- [50] World Health Organization, "Guidelines on Core Components of Infection Prevention and Control Programmes," WHO Press, Geneva, 2016.