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ASSESSMENT OF ANTIBACTERIAL PROPERTIES OF *Azadirachta indica* SEED OIL AGAINST SELECTED PHYTOPATHOGENIC BACTERIA

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ABSTRACT

The growing resistance of phytopathogenic bacteria to conventional bactericides has intensified the search for sustainable crop protection strategies. Neem (*Azadirachta indica*) seed oil is widely promoted as a botanical biopesticide with antimicrobial potential due to its complex composition and bioactive properties. However, its efficacy against phytopathogens remains poorly characterized. In this study, the antibacterial activity of neem seed oil was evaluated against four agriculturally important phytopathogenic bacteria using the broth microdilution assay. Despite its recognized bioactivity, neem seed oil did not show inhibitory or bactericidal effects against the selected strains within the tested concentration range. These findings indicate that the antimicrobial efficacy of neem-based products depends on formulation properties, chemical composition, and pathogen-specific susceptibility, highlighting the need for rigorous evaluation and optimized formulations before agricultural application.

INTRODUCTION

The rising prevalence of antimicrobial resistance among phytopathogenic bacteria poses a significant challenge for modern agriculture. Conventional disease control strategies rely primarily on synthetic pesticides and copper-based antimicrobial agents; however, their extensive and repeated use has contributed to the emergence of resistant bacterial strains, reduced treatment efficacy, and growing environmental and human health concerns [1]. These challenges have intensified the demand for sustainable, environmentally friendly alternatives in plant disease management. In this context, biopesticides derived from natural sources have gained increasing attention as promising tools for sustainable agriculture. Plant-based biopesticides are particularly attractive due to their biodegradability, chemical diversity, and broad spectrum of biological activities, including insecticidal, antifungal, and antibacterial effects [2]. Among them, neem (*Azadirachta indica*) seed oil has become one of the most extensively

studied botanical biopesticides. Its biological activity is primarily attributed to limonoids such as azadirachtin, along with other bioactive compounds, including nimbin and salannin, which contribute to its pesticidal and antimicrobial properties [3].

The antibacterial efficacy of neem seed oil against phytopathogenic bacteria remains insufficiently validated and inconsistent across studies. Neem oil is highly hydrophobic, so its biological activity may depend on formulation stability, emulsification efficiency, and the bioavailability of active constituents. Although antimicrobial activity is frequently attributed to neem-derived products, supporting evidence remains heterogeneous and often dependent on experimental design and extraction methodology. Phytopathogenic bacteria such as *Xanthomonas campestris* pv. *campestris*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pectobacterium carotovorum* subsp. *brasiliense*, and *Pseudomonas syringae* pv. *aptata* cause economically significant crop diseases worldwide and are often difficult to manage because of their adaptability and environmental persistence. Therefore, this study aimed to evaluate the antibacterial activity of neem seed oil against selected phytopathogenic bacteria of agricultural relevance and to assess its potential as a natural biopesticide for sustainable crop protection.

EXPERIMENTAL

Commercial cold-pressed neem (*Azadirachta indica*) seed oil (Eterra, TemaCo d.o.o., Serbia; origin: India) was evaluated for antibacterial activity against four agriculturally relevant phytopathogenic bacteria: *Xanthomonas campestris* pv. *campestris* (NCPPB 528), *Clavibacter michiganensis* subsp. *michiganensis* (CFBP 4999), *Pectobacterium carotovorum* subsp. *brasiliense* (PCB 2811), and *Pseudomonas syringae* pv. *aptata* (P21), belonging to the bacterial strain collection of the Department of Microbiology, University of Belgrade, Faculty of Biology. Neem oil emulsions were prepared in Mueller–Hinton broth using Tween 20 (Sigma-Aldrich, France) as an emulsifying agent. Antibacterial activity was assessed using the broth microdilution method in 96-well microplates by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Neem oil was tested over a concentration range of 0.078–100 mg/mL against bacterial inocula standardized to 1×10^6 CFU/mL. Streptomycin (concentration range 2-256 μg/mL) and tetracycline (concentration range 0.5-64 μg/mL) served as positive controls, while serial dilutions of Tween 20 (concentration range 0.0078-10%) were included as a solvent control. Following incubation at 30 °C for 24 h, bacterial viability was assessed using a resazurin-based assay, while bactericidal activity was confirmed by subculturing in Mueller–Hinton agar (TMMedia, India).

RESULTS AND DISCUSSION

The antibacterial activity of *Azadirachta indica* seed oil against selected phytopathogenic bacterial strains was evaluated using the broth microdilution method to determine the MIC and MBC. Tween 20, used as the emulsifying agent, did not exhibit antibacterial activity. As shown in Table 1, neem seed oil showed no inhibitory or bactericidal activity against the selected strains at the tested concentrations. In contrast, the positive controls showed expected antibacterial activity. Streptomycin exhibited MIC values ranging from ≤ 2 µg/mL to 8 µg/mL and MBC values from 8 µg/mL to 32 µg/mL, while tetracycline showed pronounced activity against strain NCPPB 528 (MIC ≤ 0.5 µg/mL; MBC 2 µg/mL). Strain PCB 2811 was not sensitive to either antibiotic, indicating a high level of resistance that further emphasizes the urgent need for alternative disease management strategies.

Table 1. Minimal inhibitory and minimal bactericidal concentrations of neem seed oil.

Strain designation	Neem seed oil (mg/mL)		Streptomycin (µg/mL)		Tetracycline (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC
NCPPB 528	> 100	nd	8	32	≤ 0.5	2
CFBP 4999	> 100	nd	8	16	> 64	nd
PCB 2811	> 100	nd	> 256	nd	> 64	nd
P21	> 100	nd	≤ 2	8	2	32

nd- not determined in the tested concentration range

These findings contrast with several reports describing the antibacterial effects of *Azadirachta indica* extracts and formulations against certain phytopathogenic *Xanthomonas*, *Pseudomonas*, and *Agrobacterium* species [4-5], underscoring that the antimicrobial efficacy of neem-derived products is highly dependent on the extraction method, formulation, and chemical composition. Indeed, antibacterial efficacy appears to vary among neem plant parts, with leaf and bark extracts generally showing stronger activity than seed and fruit extracts [6]. Additionally, previous greenhouse and field studies suggest that neem oil formulations may contribute to disease suppression through indirect mechanisms, including modulation of plant defense responses and interference with pathogen colonization [7].

The absence of measurable antibacterial activity observed in this study may be attributed to the limited bioavailability and dispersion of hydrophobic neem oil constituents in aqueous culture systems, thereby reducing their interaction with bacterial cells. In addition, formulation-related factors and the intrinsic resistance mechanisms of phytopathogenic bacteria may further influence the antimicrobial efficacy of neem-based products. Collectively, these findings highlight the complexity of neem-based antimicrobial activity and underscore the

need for detailed chemical characterization, optimized formulations, and evaluation of isolated bioactive constituents against a broader range of phytopathogens.

CONCLUSION

Under the experimental conditions used, *Azadirachta indica* seed oil did not show direct antibacterial activity against the phytopathogenic bacteria tested. These findings underscore the importance of optimizing formulations and conducting standardized biological evaluations in developing evidence-based neem-derived biopesticides for sustainable agriculture.

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