

الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

Ferhat Abbas University, Sétif 1
Faculty of Nature and Life
Sciences



جامعة فرحات عباس، سطيف 1
كلية علوم الطبيعة والحياة

Department of Microbiology

N°...../SNV/2025

THESIS

Presented by

BELLA Dhiaa eddine Anis

HAMMOUCHE Aya

HEMICI Samie Elhak

In view of obtaining the degree of

MASTER IN BIOLOGICAL SCIENCES

Field: **Biology**

Specialty: **Applied Microbiology**

Topic

**Essential Oils and Their Nanoemulsions as
Lemon Bioconservators Against *Penicillium spp.*:
An Integrated *In Vitro*, *In Vivo*, and *In Silico*
Evaluation.**

Presented publicly in: 22/06/2025

Jury:

President

Mr Bachir Adel

MCB-UFAS 1

Supervisor

Mr Bencheikh Amor

MCA-UFAS 1

Examinator

Mr Yahiaoui Bilal

Pr-UFAS 1

Laboratory of Applied Microbiology

Acknowledgments

First and foremost, all praise and thanks are due to **ALLAH**, for granting us the patience, strength, and unwavering determination to embark upon and successfully complete this work. Without divine guidance, this endeavour would not have been possible.

We extend our deepest gratitude to our esteemed supervisor, **Mr. Bencheikh**, whose tireless **effort, invaluable guidance, and constant encouragement** illuminated our path throughout this research. His profound insights and unwavering support were instrumental in shaping this thesis.

Our sincere appreciation goes to the distinguished members of the jury, **Mr. Yahiaoui and Mr. Bachir**, for honouring us with their presence and taking the time to evaluate our work. Their expertise and thoughtful feedback are greatly valued.

We would also like to express our heartfelt thanks to **Mrs Merihane Gharzouli** and **Mrs Lamia Laribi** for their invaluable help and active participation, which significantly contributed to the development of this thesis. Their support was truly a cornerstone of our efforts.

Finally, a special note of gratitude is etched for **Mrs Boudechicha Amel**. Her boundless dedication, unwavering support, and every gesture, big and small, throughout this process were truly exceptional and will forever be cherished.

To all those who participated, near and far, in the development of this work, we extend our deepest thanks for your invaluable contributions.

Dedication

I begin by offering my deepest gratitude to God Almighty for granting me the strength, knowledge, and perseverance to complete this work.

My sincere thanks go to my esteemed professors for their unwavering support and to everyone who assisted with this work through technical support, valuable discussions, or encouragement; your contributions were invaluable.

To my extraordinary partners, **Anis** and **Samie**, I am profoundly grateful for your unwavering dedication, tireless efforts, and the boundless creativity you brought to every aspect of this project. Your insightful contributions, collaborative spirit, and true partnership have been absolutely integral to the success of this work, and frankly, without you, this work would never have been realized.

To my pillars of strength, my beloved **mother** and **father**, I offer my eternal and deepest gratitude. Your unconditional love, endless sacrifices, and unwavering belief in my potential have been the bedrock of my entire life.

I dedicate this work to the loving memory of my dear grandmother. How I wish you were here to see this moment. May her soul, and the souls of all my departed family, rest in eternal peace.

To my brothers and sisters, especially Nour and Ilham, thank you for always being by my side. Your steadfast support, encouragement, and understanding through every challenge have been a constant source of comfort and motivation. Knowing I had your unwavering backing made all the difference.

Finally, to my soulmate, **Meriem**, and all my dear friends **Amel**, **Amina**, **Yasmine**, **Rahma**, and **Meriem**, I am deeply thankful for your companionship, encouragement, and the joy you have brought to this journey.

My gratitude extends to everyone else who offered their help and support along this work. Your kindness did not go unnoticed.

Hammouche Aya

Dedication

With deep gratitude and immense joy, I dedicate this work to those who have been my unwavering pillars throughout this journey.

First and foremost, all praise and thanks be to God, whose grace granted us the strength, perseverance, and wisdom to overcome every challenge.

To my beloved parents—their love, sacrifice, and unwavering support have been the foundation of all my achievements. This success is as much yours as it is mine.

To my dear sisters, thank you for your constant encouragement, understanding, and belief in me.

To my incredible partners **Anis** and **Aya** in this endeavour— thank you for your hard work, resilience, and dedication. You carried every challenge with grace and made even the hardest parts feel effortless. I'm truly honoured to have shared this path with you.

To the friends who became family, their companionship and support brought light and laughter during the most demanding times.

Finally, to **everyone who contributed**, directly or indirectly, to the completion of this work, your assistance, insights, and guidance were deeply appreciated. And thanks to **everyone who believed in us** and wished to see us succeed.

Hemici Samie Elhak

Dedication

Honestly, I have to start by thanking me, myself, and I – but really, all the credit goes to my wonderful **parents**, particularly **my mom**, for getting me to where I am today. I was incredibly lucky to have my **brothers** and **friends**, as they provided the ideal environment for me to develop into the smart, creative, competitive, and ambitious man I am.

Furthermore, I just wanna say that's everything destined to be, from the day god created the earth I've been destined to do what I did, every step, every word every word I said has been a meticulously choreographed movement in my grand, inevitable ascent. I didn't achieve this; I merely unveiled what was always meant to be, and what will continue to unfold into an empire of my making, far beyond any mortal comprehension.

“I cannot be mistaken. What I do and say is historical”

Bella Anis

Abstract

This study aimed to evaluate the efficacy of essential oils (EOs) and their nano-emulsions (NEs) as natural alternatives for managing post-harvest blue mold in lemons, with specific focus on their antifungal properties and possible practical application. Essential oils were extracted from *Pimpinella anisum* (PEO) with a yield of 2.7% and *Saccocalyx satureioides* (SEO) with a yield of 2%. Nano-emulsions were successfully formulated; SNEO exhibited a mean particle size of 111.8 nm and a zeta potential of -45.6 mV, while PNEO had a size of 263.6 nm and a zeta potential of -33.3 mV, both demonstrating good kinetic stability. Biological activity assessments revealed significant findings. The *in vitro* antifungal tests against *Penicillium italicum*, nano-emulsions showed superior activity, with PNEO demonstrating an inhibition diameter of 33 mm compared to 15 mm for neat PEO. Similarly, MIC values against *P. italicum* were significantly lower for nano-emulsions (0.0781% v/v for both PNEO and SNEO) compared to their neat essential oil counterparts (0.1562% v/v). For antioxidant activity, SEO and SNEO exhibited strong DPPH radical inhibition, with IC₅₀ values of 3.45% and 3.11% respectively, whereas PEO and PNEO showed a very weak activity. *In vivo* application on lemons demonstrated that SNEO and SEO maintained superior preservative capabilities over 21 days. Treated lemons largely retained their initial pH (e.g., 3.05-3.09) compared to the untreated control (approximately 3.5). Total Soluble Solids (TSS) generally showed no significant change, while SNEO-treated lemons maintained Total Acidity at 0.454%, close to the fresh fruit's 0.46%. Furthermore, an *in silico* study further identified potent antifungal compounds from the EOs, such as Rotundifolone and thymol, with strong binding affinities to fungal targets. These results collectively indicate significant progress towards achieving the study's objectives of developing effective and natural post-harvest disease management strategies.

Keywords: Antifungal activity, essential oils, *in silico* molecular docking, nano-emulsions, *Penicillium italicum*, post-harvest diseases.

ملخص

هدفت هذه الدراسة إلى تقييم فعالية الزيوت الأساسية (EOs) ومستحلباتها النانوية (NEs) كبدايل طبيعية لمكافحة العفن الأزرق في الليمون بعد الحصاد، مع التركيز بشكل خاص على خصائصها المضادة للفطريات وإمكانية تطبيقها العملي. تم استخلاص الزيوت الأساسية من نبات *Pimpinella anisum* (PEO) بمردود 2.7% ومن نبات *Saccocalyx satureioides* (SEO) بمردود 2%. تم تحضير المستحلبات النانوية بنجاح؛ حيث أظهر SNEO متوسط حجم جسيمات قدره 111.8 نانومتر وجهد زيتا قدره -45.6 ملي فولت، بينما بلغ حجم PNEO 263.6 نانومتر بجهد زيتا قدره -33.3 ملي فولت، وكلاهما أظهر استقراراً حركياً جيداً. كشفت تقييمات النشاط البيولوجي عن نتائج مهمة في الاختبارات المضادة للفطريات في المختبر ضد *Penicillium italicum*. أظهرت المستحلبات النانوية نشاطاً فائقاً، حيث أظهر PNEO قطر تثبيط بلغ 33 ملم مقارنة بـ 15 ملم لـ PEO النقي. وبالمثل، كانت قيم التركيز المثبط الأدنى (MIC) ضد *P. italicum* أقل بكثير للمستحلبات النانوية (0.0781% حجم/حجم) لكل من PNEO و SNEO مقارنة بنظيراتها من الزيوت الأساسية النقية (0.1562% حجم/حجم). أما بالنسبة لنشاط مضادات الأكسدة، فقد أظهر SEO و SNEO تثبيطاً قوياً لجذور DPPH الحرة، بقيم IC₅₀ بلغت 3.45% و 3.11% على التوالي. على العكس، أظهر PEO و PNEO نشاطاً ضعيفاً جداً. أظهر التطبيق على حبات الليمون أن SNEO و SEO حافظا على قدرات حفظ فائقة على مدار 21 يوماً. احتفظ الليمون المعالج إلى حد كبير برقم الحموضة الأولي (3.05-3.09) مقارنة بالشواهد غير المعالجة (حوالي 3.5). لم تظهر المواد الصلبة الذائبة الكلية (TSS) عمومًا تغييراً كبيراً، بينما حافظ الليمون المعالج بـ SNEO على الحموضة الكلية عند 0.454%، وهو قريب من للفاكهة الطازجة (0.46%). علاوة على ذلك، حددت دراسة الحاسوبية (*in silico*) مركبات قوية مضادة للفطريات من الزيوت الأساسية، مثل Rotundifolone والثيمول، ذات تقارب ارتباط قوي بالأهداف الفطرية. تشير هذه النتائج بشكل جماعي إلى تقدم كبير نحو تحقيق أهداف الدراسة في تطوير استراتيجيات فعالة وطبيعية لإدارة أمراض ما بعد الحصاد عند الحمضيات خاصة.

الكلمات المفتاحية: زيوت أساسية؛ مستحلبات نانوية؛ نشاط مضاد للفطريات؛ أمراض ما بعد الحصاد؛ *Penicillium italicum*؛ الإرساء الجزيئي الحاسوبي (*in silico*).

Résumé

Cette étude visait à évaluer l'efficacité des huiles essentielles (HE) et de leurs nano-émulsions (NE) en tant qu'alternatives naturelles pour la gestion de la moisissure bleue post-récolte chez les citrons, en se concentrant spécifiquement sur leurs propriétés antifongiques et leur application pratique. Des huiles essentielles ont été extraites de *Pimpinella anisum* (HEP) avec un rendement de 2,7 % et de *Saccocalyx satureioides* (HES) avec un rendement de 2 %. Des nano-émulsions ont été formulées avec succès; la NESH a présenté une taille moyenne de particule de 111,8 nm et un potentiel zêta de -45,6 mV, tandis que la NEHP avait une taille de 263,6 nm et un potentiel zêta de -33,3 mV, les deux démontrant une bonne stabilité cinétique. Les évaluations de l'activité biologique ont révélé des résultats significatifs. Lors des tests antifongiques *in vitro* contre *Penicillium italicum*, les nano-émulsions ont montré une activité supérieure, la NEHP démontrant un diamètre d'inhibition de 33 mm comparativement à 15 mm pour l'HEP pure. De même, les valeurs de CMI (Concentration Minimale Inhibitoire) contre *P. italicum* étaient significativement plus faibles pour les nano-émulsions (0,0781 % v/v pour la NEHP et la NESH) par rapport à leurs contreparties d'huiles essentielles pures (0,1562 % v/v). Concernant l'activité antioxydante, l'HES et la NESH ont montré une forte inhibition du radical DPPH, avec des valeurs de CI50 de 3,45 % et 3,11 % respectivement, tandis que l'HEP et la NEHP ont montré une très faible activité. L'application *in vivo* sur les citrons a démontré que la NESH et l'HES maintenaient des capacités de conservation supérieures sur 21 jours. Les citrons traités ont largement conservé leur pH initial (par exemple, 3,05-3,09) comparativement au témoin non traité (environ 3,5). Les Solides Solubles Totaux (SST) n'ont généralement montré aucun changement significatif, tandis que les citrons traités à la NESH ont maintenu une Acidité Totale à 0,454 %, proche de celle du fruit frais (0,46 %). De plus, une étude *in silico* a permis d'identifier des composés antifongiques puissants issus des HE, tels que le Rotundifolone et le thymol, avec de fortes affinités de liaison aux cibles fongiques. Ces résultats indiquent collectivement des progrès significatifs vers l'atteinte des objectifs de l'étude, à savoir le développement de stratégies efficaces et naturelles de gestion des maladies post-récolte.

Mots-clés : Activité antifongique, amarrage moléculaire *in silico*, huiles essentielles, maladies post-récolte, nano-émulsions, *Penicillium italicum*.