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Thèse

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Etude comparative de l'efficacité des extraits aqueux et huiles essentielles de quelques plantes spontanées et cultivées de la région d'Ouargla (Sahara septentrional) sur des champignons phytopathogènes isolés à partir de la pomme de terre (*Solanum tuberosum*).

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Fatma Zohra

Résumé

Etude comparative de l'efficacité des extraits aqueux et huiles essentielles de quelques plantes spontanées et cultivées de la région d'Ouargla (Sahara septentrional) sur des champignons phytopathogènes isolés à partir de la pomme de terre (*Solanum tuberosum*).

Notre travail a consisté dans un premier temps à isoler et identifier les champignons phytopathogènes présents sur les tubercules de pomme de terre après récolte, provenant de la région d'El Oued ; puis d'étudier la composition chimique des huiles essentielles et des extraits aqueux des parties aériennes de *Mentha spicata* L*, Mentha pepirita* L*, Mentha pulegium* L et *Ocimum basilicum* L, afin d'évaluer leurs propriétés antifongiques. Les résultats de l'analyse de l'ADNr 18S ont confirmé l'identification des champignons infestant les tubercules comme étant *Fusarium proliferatum, Alternaria alternaria, Rhizoctonia solani* et *Wickerhamomyces anomalus.* Les huiles essentielles extraites des plantes ont été analysées par GS-MS, indiquant que le (-)- Carvone constitue le composant principal de *M. spicata* (41.66 %), de *M. piperita* (48,74%) et de *M. Pulegium* (30,89%). *O.basilicum* contient principalement du Linalol (26,16%). *M. spicata* présente un effet antifongique de 100% contre *F. proliferatum* à la concentration 0.90%, et 0.95% pour *A. alternata, R.solani* et *W. anomalus*. Concernant *M. pepirita,* une inhibition totale (100%) a également été obtenue sur toutes les souches, aux concentrations 0.95% et 1%, à 0.87% pour *W. anomalus* et à 0.90% pour *A.alternata*. L'huile essentielle de *M. pulegium* a eu un effet fongicide à la concentration 0,97% sur *A. alternata* et *F. proliferatum*, à 0,95% sur *W. anomalus* et *R. solani*. Concernant *O. bacilicum*, une inhibition totale (100%) de la croissance mycélienne à la concentration 0.97% a été observée sur toutes les souches, sauf *W. anomalus* pour lequel l'effet fongicide a été atteint à la concentration 1%. Les tests phytochimiques effectués sur les extraits aqueux ont mis en évidence la présence de divers composés chimiques tels que les tanins, les flavonoïdes et les saponines. La teneur en composés phénoliques totaux des différents extraits était comprise entre 3,2770 et 11,1248 mg, tandis que la teneur en flavonoïdes totaux variait entre 0,8961 et 2,3122 mg. L'analyse des extraits aqueux par HPLC a révélé la présence de divers composés chimiques tels que la caféine, l'acide gallique et l'acide acétylsalicylique. Les résultats ont montré un fort potentiel d'activité antifongique. Aux concentrations 1,5%, 2%, 3,5% et 4%, l'extrait aqueux de *M. spicata* a présenté un effet antifongique de 100% sur tous les champignons. Concernant *M. pepirita*, l'extrait à 1%, a provoqué une inhibition totale de 100% chez *A. alternata* et *W. anomalus*, et à 4% chez *F. proliferatum* et *R. solani*. Nous avons observé un effet inhibiteur fongicide (100 %) à 1,5 % chez *A. alternata* et *W. anomalus*, et à 3,5% chez *F. proliferatum* et *R. solani* pour l'extrait de *M. pulegium*. L'extrait d*'O. bacilicum* a entrainé une inhibition totale (100%) aux concentrations 2%, 2.5%, 5.5% et 7.5% chez *W. anomalus*, *A. alternata, F.proliferatum* et *R. solani* respectivement.

Mots clés : Pomme de terre, champignons phytopathogènes, menthe, basilic, activité antifongique.

ملخص

دراسة مقارنة لفعالية المستخلصات المائية والزيوت الأساسية لبعض النباتات العفوية والمزروعة في منطقة ورقلة (شمال الصحراء) على الفطريات الممرضة للنباتات المعزولة من البطاطس.(Solanum tuberosum)

في البداية، تركز عملنا على عزل وتحديد الفطريات المسببة للأمراض النباتية الموجودة على درنات البطاطس بعد الحصاد من منطقة الوادي. ثم قمنا بدراسة التركيب الكيميائي للزيوت الأساسية والمستخلصات المائية للأجزاء الهوائية من *Mentha pulegium* L, *Mentha piperita* L, *Mentha spicata* L و *Ocimum basilicum* L لتقييم خصائصها المضادة للفطريات. أكدت نتائج تحليل الحمض النووي الريبي الريبوسومي 18 S تحديد الفطريات المصابة للدرنات وهي *Rhizoctonia solani*, *Alternaria alternaria*, *Fusarium proliferatum* و *Wickerhamomyces anomalus*. تم تحليل الزيوت الأساسية المستخرجة من النباتات بواسطةGS-MS ، مشيرةً إلى أن (-)-كارفون هو المكون الرئيسي لـ(41.66%) *M. spicata* ، (48.74%) *M. piperita* و *M. pulegium* (30.89%).يحتوي *O. basilicum* بشكل رئيسي على لينالول (26.16%). أظهر *M. spicata* تأثيرًا مضادًا للفطريات بنسبة 100% ضد *F. proliferatum* عند تركيز 0.90% و 0.95% ضد *A. alternata, R. solani* و *W. anomalus*.بالنسبة لـ*M. piperita* ، تم الحصول أيضًا على تثبيط كلي (100%) لجميع السلالات عند تركيزات 0.95% و 1%، 0.87% لـ *W. anomalus* و 0.90% لـ *A. alternata*. كان لزيت *M. pulegium* الأساسي تأثير فطري عند تركيز 0.97% على *A. alternata* و*F. proliferatum* ، و 0.95% على *W. anomalus* و *R. solani*.بالنسبة ل *O. basilicum* ، لوحظ تثبيط كلي (100%) لنمو الفطريات عند تركيز 0.97% لجميع السلالات، باستثناء *W. anomalus* حيث تم الوصول إلى التأثير الفطري عند تركيز 1 %. أظهرت الاختبارات الكيميائية النباتية على المستخلصات المائية وجود مركبات كيميائية متنوعة مثل التانينات، الفلافونويدات والصابونينات. تراوح محتوى المركبات الفينولية الكلية في المستخلصات المختلفة بين 3.277 و11.124 مجم، بينما تراوح محتوى الفلافونويدات الكلية بين 0.896 و2.312 مجم. كشف تحليل HPLC للمستخلصات المائية وجود مركبات كيميائية متنوعة مثل الكافيين، حمض الجاليك وحمض الأسيتيل ساليسيليك. أظهرت النتائج وجود إمكانيات كبيرة للنشاط المضاد للفطريات. عند تركيزات 1.5%، 2%، 3.5% و4%، أظهر المستخلص المائي *M.spicata* تأثيرًا مضادًا للفطريات بنسبة 100% على جميع الفطريات. بالنسبة ل *M. piperita* ، تسبب المستخلص بتركيز 1% في تثبيط كلي بنسبة 100% ل *A. alternata*و *W. anomalus* ، وعند 4% ل *F. proliferatum* و *R. solani*. لاحظنا تأثيرًا فطريًا مثبطًا (100%) عند 1.5% ل *A. alternata* و*W. anomalus* ، وعند 3.5% ل *F. proliferatum* و *R. solani* لمستخلص *M. pulegium*.أدى مستخلص *O. basilicum* إلى تثبيط كلي (100%) عند تركيزات 2%، 2.5%، 5.5% و7.5% ل *W. anomalus* ، *A. alternata*، *F. proliferatum* و *R. solani* على التوالي.

الكلمات المفتاحية: البطاطس، الفطريات المسببة للأمراض النباتية، النعناع، الريحان، النشاط المضاد للفطريات.

Abstract

Comparative study of the effectiveness of aqueous extracts and essential oils from some spontaneous and cultivated plants in the Ouargla region (northern Sahara) on phytopathogenic fungi isolated from potatoes (*Solanum tuberosum*).

Initially involved isolating and identifying the phytopathogenic fungi present on post-harvest potato tubers from the El Oued region. We then studied the chemical composition of essential oils and aqueous extracts from the aerial parts of *Mentha spicata* L, *Mentha piperita* L, *Mentha pulegium* L, and *Ocimum basilicum* L to evaluate their antifungal properties. The results of the 18S rDNA analysis confirmed the identification of the fungi infecting the tubers as *Fusarium proliferatum*, *Alternaria alternaria*, *Rhizoctonia solani*, and *Wickerhamomyces anomalus*. The essential oils extracted from the plants were analyzed by GC-MS, indicating that (-)- Carvone is the main component of *M. spicata* (41.66%), *M. piperita* (48.74%), and *M. pulegium* (30.89%). *O. basilicum* primarily contains Linalool (26.16%). *M. spicata* exhibited 100% antifungal effect against *F. proliferatum* at a concentration of 0.90%, and 0.95% against *A. alternata*, *R. solani*, and *W. anomalus*. For *M. piperita*, complete inhibition (100%) was also obtained on all strains at concentrations of 0.95% and 1%, 0.87% for *W. anomalus*, and 0.90% for *A. alternata*. The essential oil of *M. pulegium* had a fungicidal effect at 0.97% on *A. alternata* and *F. proliferatum*, and at 0.95% on *W. anomalus* and *R. solani*. For *O. basilicum*, total inhibition (100%) of mycelial growth at 0.97% was observed on all strains except for *W. anomalus*, where the fungicidal effect was achieved at 1%. Phytochemical tests on the aqueous extracts highlighted the presence of various chemical compounds such as tannins, flavonoids, and saponins. The total phenolic content of the different extracts ranged from 3.277 to 11.124 mg, while the total flavonoid content varied from 0.896 to 2.312 mg. HPLC analysis of the aqueous extracts revealed the presence of various chemical compounds, including caffeine, gallic acid, and acetylsalicylic acid. The results showed a strong potential for antifungal activity. At concentrations of 1.5%, 2%, 3.5%, and 4%, the aqueous extract of *M. spicata* exhibited 100% antifungal effect on all fungi. For *M. piperita*, the extract at 1% caused complete inhibition (100%) in *A. alternata* and *W. anomalus*, and at 4% in *F. proliferatum* and *R. solani.* We observed a fungicidal inhibitory effect (100%) at 1.5% in *A. alternata* and *W. anomalus*, and at 3.5% in *F. proliferatum* and *R. solani* for the extract of *M. pulegium*. The extract of *O. basilicum* resulted in total inhibition (100%) at concentrations of 2%, 2.5%, 5.5%, and 7.5% in *W. anomalus*, *A. alternata*, *F. proliferatum*, and *R. solani* respectively.

Keywords: Potato, antifungal activity, mint, basil, phytopathogenic fungi

Liste des abréviations

PDA	Potatos Dextrose Agar
ADN	Acide désoxyribonucléique
ADNr	Acide désoxyribonucléique ribosomique
ITS	Espaceur transcrit interne ribosomal nucléaire
NaOCl	Hypochlorite de Sodium
EF	Elongation factor 1-alpha
PCR	Polymerase Chain Reaction
$MgCl_2$	Chlorure de magnésium
BLAST	Basic local alignment search tool
NCBI	Centre National d'Information sur la Biotechnologie
GS-MS	Chromatographie en phase gazeuse, couplée à la spectrométrie de masse
NIST	Institut National des Normes et de la Technologie
HE	Huile essentielle
HPLC	Chromatographie en phase liquide à haute performance
FAO	Organisation des Nations Unies pour l'alimentation et l'agriculture
Na_2CO_3	Carbonate de sodium
TPC	Contenu phénolique total
TFC	Teneur totale en flavonoïdes
GAE	Equivalents d'acide gallique
DW	Poids sec
QE	Equivalents de quercétine
CMI	Concentrations Minimales Inhibitrices
MS	Spectrométrie de masse

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SOMMAIRE

Introduction Générale

INTRODUCTION GENERALE

La pomme de terre est considérée comme l'une des cultures les plus importantes à l'échelle mondiale, après le blé, le riz et le maïs **(Benkeblia, 2020).** Selon **Devaux *et al.* (2020),** la superficie mondiale de production de pommes de terre est estimée à 19 millions d'hectares, ce qui équivaut à environ 378 millions de tonnes.

D'après les données récentes, les maladies causées par les champignons ont principalement affecté les cultures vivrières essentielles telles que le riz, le blé, le maïs et la pomme de terre, entraînant des pertes économiques de plusieurs milliards de dollars **(Peng *et al.*, 2020).**

Selon **Shuping et Eloff (2017),** il y avait environ 8 000 espèces fongiques différentes qui étaient responsables d'environ 100 000 maladies différentes des plantes. Au cours des dernières années, une augmentation du nombre de champignons responsables de maladies des plantes à travers le monde a été signalée, atteignant plus de 19 000 **(Jain *et al.*, 2019).**

Les champignons ont développé diverses méthodes pour coloniser les plantes ; ils sont toujours dormants et pourraient survivre jusqu'à ce que des conditions propices à leur prolifération soient créées **(Doehlemann *et al.*, 2017 ; Jain *et al.*, 2019).** Les champignons génèrent des toxines qui contribuent à la croissance des maladies des plantes. Les métabolites secondaires de ces toxines, qui possèdent un poids moléculaire faible, peuvent entraîner des symptômes particuliers tels que la chlorose, la nécrose, le flétrissement, l'inhibition de la croissance et les taches sur les feuilles **(Proctor *et al.*, 2018 ; Jajić *et al.*, 2019).**

Les maladies fongiques des plantes causent des pertes significatives en termes de quantité et de qualité dans la production agricole. Selon **Carling *et al* (1989),** *Rhizoctonia solani* est l'un des agents pathogènes qui cause des maladies des cultures qui affectent les racines et les tubercules, entraînant des pertes importantes. D'autre part, *Alternaria alternata* peut être responsable de diverses maladies des plantes à travers le monde, comme la tache brune **(Garganese *et al.*, 2016)** et la pourriture du cœur **(Aloi *et al.*, 2021 ; Mincuzzi *et al.*, 2022 ; Manjunatha *et al.*, 2022).**

Aujourd'hui, les fongicides artificiels sont couramment employés afin de préserver les plantes de ces agents pathogènes. Toutefois, leur fabrication représente un coût considérable et ils peuvent laisser des résidus toxiques sur les surfaces traitées. En outre, avec la constante utilisation de fongicides synthétiques de la même catégorie, plusieurs produits

phytopharmaceutiques ont perdu leur efficacité en raison de la résistance développée par les agents pathogènes fongiques **(Cantrell *et al*., 2012).** Par ailleurs, leur faible sélectivité et leur non-biodégradabilité les rendent dangereux pour l'environnement et les êtres humains, et ils peuvent également entraîner une résistance des micro-organismes, ce qui peut entraîner des problèmes de sécurité alimentaire **(Ahmad *et al*., 2020).**

Il est donc urgent de développer de nouveaux modèles de traitements contre les infections fongiques. En tant que stratégies alternatives, les fongicides botaniques ont suscité l'intérêt de nombreux chercheurs ces dernières années ; plusieurs recherches ont prouvé que les produits phytochimiques issus des plantes ont des propriétés fongicides **(Bhandari *et al.*, 2021).**

Selon **Suteu *et al*. (2020),** il est possible de fournir des substances organiques qui peuvent être classées en métabolites primaires (protéines, glucides et graisses) ou en métabolites secondaires (terpènes, stéroïdes, anthocyanes, anthraquinones, phénols, alcaloïdes, etc.). Il a été démontré que de nombreuses plantes aromatiques dont leurs huiles essentielles et extraits aqueux, présentent des propriétés antimycotiques et peuvent potentiellement servir d'agents antifongiques.

De par sa situation géographique, l'Algérie offre une végétation riche et diversifiée. De nombreuses plantes aromatiques y poussent. L'intérêt pour ces plantes n'a cessé de croître ces dernières années **(Lorenzo *et al*., 2002).** Parmi ces plantes, la famille des Lamiacées comprend environ 7 000 espèces réparties dans plus de 250 genres, répartis dans le monde **(Napoli *et al*., 2020),** dont 146 espèces réparties dans 28 genres en Algérie **(Quezel et Santa, 1963).** Les espèces de cette famille contiennent des substances pharmacologiquement actives qui sont également utilisées dans les cosmétiques, les aliments et les pesticides **(Lee *et al*., 2011 ; Khodja *et al.*, 2014).**

Mentha est un genre bien connu de la famille des Lamiacées, qui comprend environ 25 espèces et hybrides. Il joue un rôle important tant en pharmacopée que dans le domaine culinaire

Originaire d'Europe et d'Asie, elle est couramment utilisée pour ses feuilles aromatiques et parfumées **Maxia *et al*. (2008).**

Pour ces diverses raisons, le choix du matériel végétal s'est orienté vers quelques espèces de la famille des Lamiacées afin de tester leur pouvoir antifongique sur des champignons infestant la pomme de terre.

Dans ce contexte, nous avons isolé et identifié des champignons phytopathogènes à partir de tubercules de pomme de terre de la région d'Oued Souf et d'évaluer l'activité antifongique d'extraits aqueux et huiles essentielles de quelque plantes médicinales et aromatiques, cultivées dans le Sud algérien.

Structure de la thèse : Le manuscrit de la thèse est composé de trois chapitres et est ainsi structuré : Introduction générale qui consiste en une brève revue de la littérature sur l'importance de la pomme de terre et les champignons responsables des maladies ainsi que l'importance de la famille des Lamiacées et les objectifs.

Plan expérimental qui représente les différents dispositifs expérimentaux adoptés lors de la réalisation des différentes parties de notre travail.

Chapitre I : dans lequel sont présentés les résultats et les discussions des travaux de recherche sur l'isolement et l'identification des champignons phytopathogénes.

Chapitre II : consacré à l'évaluation de l'activité antifongique des extraits aqueux de quatre plantes sur les champignons phytopathogènes isolés.

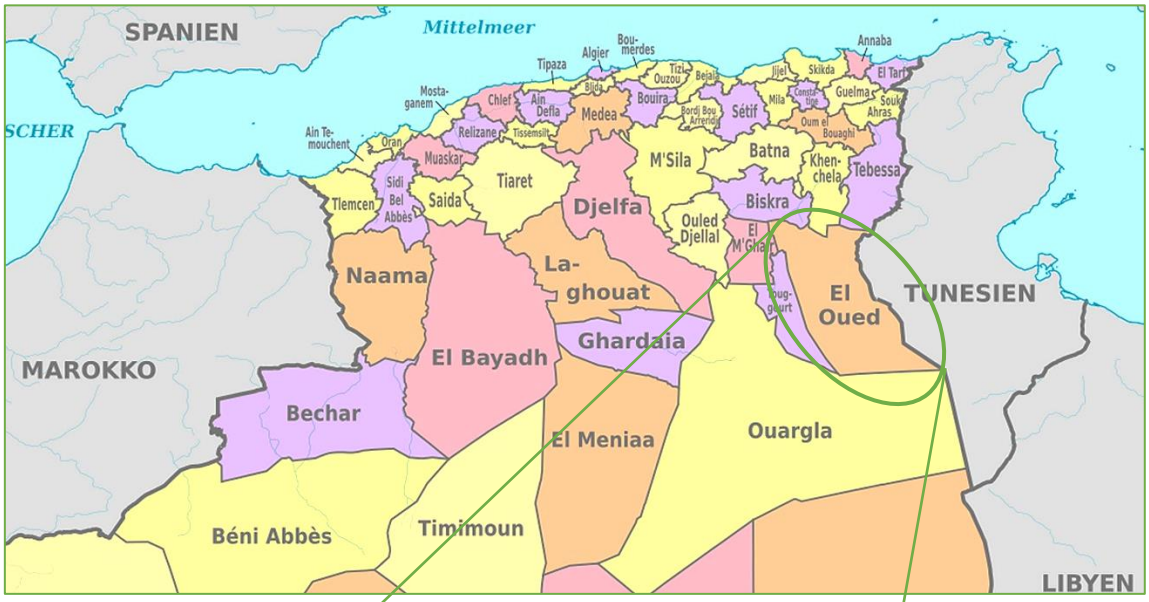
Chapitre III : rapporte les résultats et les discussions de l'activité antifongique des huiles essentielles de quatre plantes sur les champignons phytopathogènes.

Conclusion : qui vient clôturer le travail et annoncer les perspectives.

Il est à noter que la présente thèse est sous forme de thèse-Articles. Elle débute par une introduction générale. Chaque chapitre est représenté par un article scientifique paru dans une revue de classe A ou B. A la fin, une conclusion générale clôture les travaux réalisés et publiés.

CHAPITRE I

Identification et caractérisation de quelques champignons phytopathogènes de la pomme de terre (*Solanum tuberosum*) en post- récolte dans la région d'El Oued (Sahara septentrional Est Algérien)



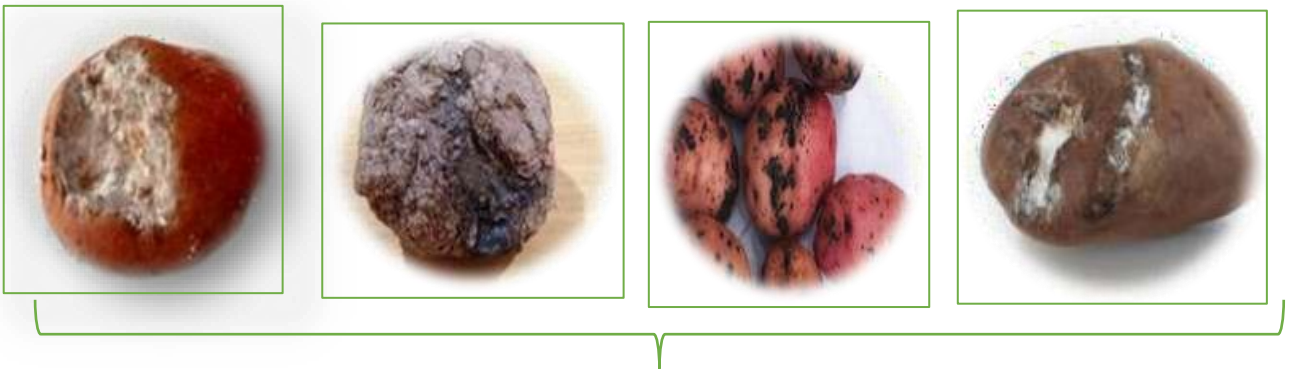
Echantillonage de pomme de terre

Symptôme 1: **Pourriture**

Symptôme 2: **Chancre**

Symptôme 3: **Galle noire**

Symptôme 4: **Flétrissement**



Isolement des champignons phytopathogènes à partir de tubercules de pomme de terre

Identification macroscopique

Identification microscopique

PCR

Figure 1. Dispositif expérimental du Chapitre 1.

Identification and Characterization of Some Phytopathogenic Fungi in Post-Harvest Potato (*Solanum tuberosum*) in the El Oued Region (Eastern Northern Sahara, Eastern Algeria) (Article publié en janvier 2024 dans la revue "International Journal of Health Sciences")

Chapitre I : Identification et caractérisation de quelques champignons phytopathogènes de la pomme de terre (*Solanum tuberosum*) en post-récolte dans la région d'El Oued (Sahara septentrional Est Algérien)

Le présent chapitre est consacré à l'isolement et à l'identification des différents champignons phytopathogènes présents sur les tubercules de pomme de terre après récolte, dans la région d'El Oued (Sahara Est algérien). La méthodologie adoptée pour la réalisation de cette partie de travail est résumée dans la **Figure 1**.

En 2019 et 2021, des tubercules de pomme de terre présentant des symptômes sous forme de taches brunes, de lésions nécrotiques foncées et de sclérotes et flétrissement **(photo 1, article)** ont été collectés à El Oued (Sud-est algérien). Nous avons utilisé le milieu de culture Potatos Dextrose Agar (PDA), qui est généralement préconisé pour la recherche et le dénombrement des moisissures, ainsi que pour le maintien des souches collectées puis repiquées **(Botton *et al*., 1990).** L'examen macroscopique a été réalisé à l'œil nu et sous la loupe binoculaire. L'examen microscopique a permis de déterminer certaines caractéristiques morphologiques telles que le type de spores et la forme du thalle.

Les colonies de *Fusarium poliferatum* ont rapidement développé un mycélium aérien blanc puis devenu violet dans l'ancienne culture. Ceci est dû à la production d'un pigment violet clair après 7 jours de culture à 25°C, avec un aspect floconneux couvrant toute la boite. Les résultats de l'examen microscopique ont révélé que *F. proliferatum* possédait un grand nombre de petites microconidies sans septa **(Figure 2, article).**

Notre résultat montre une colonie dont la couleur varie du vert olive à noire **(Figure 3, article).** La majorité des colonies ont un aspect duveteux ou cotonneux et une vitesse de croissance particulièrement lente sur le milieu PDA. L'observation microscopique des colonies d'*Alternaria alternata* a révélé une sporulation significative, avec de nombreuses conidies pluricellulaires, verdâtres entourant les mycéliums.

La croissance mycélienne présente un aspect blanchâtre et couvre rapidement la totalité de la boite **(Figure 4, article).** Elle devient progressivement brune avec le temps et

présente des sclérotes bruns foncé. L'observation microscopique des colonies pures a révélé les caractéristiques morphologiques typiques du *Rhizoctonia solani*, à savoir des hyphes cloisonnés incolores, un motif de ramification à angle droit.

Les colonies de *Wickerhamomyces anomalus* blanches, isolées ont présenté un développement rapide à aspect cotonneux. Au microscope, les cellules étaient rondes, bourgeonnantes, dispersées et reproductrices **(Figure 5, article).**

Concernant l'identification par voie génétique, celle-ci a effectuée au niveau de laboratoire de recherche : Gene Life Sciences **(Université de Sidi Bel Abbes).**

La région de séquence transcrite interne (ITS) des champignons a été amplifiée par PCR et soumise aux données de séquençage dans la genbank NCBI (le numéro d'accès GenBank est OQ606246.1, OQ860003.1, OQ771178.1 et OQ606247.1). Une recherche BLASTA (https://blast.ncbi.nlm.nih.gov/Blast.cgi Blast) des produits de PCR séquencés a révélé que les souches de champignons isolés étaient *Fusarium proliferatum* avec une identité de 99.77 %, *Alternaria alternata* et *Rhizoctonia solani* avec une identité de 100 % et *Wickerhamomyces anomalus* avec une identité de 99.83%.

A notre connaissance il s'agit du premier rapport confirmant diverses maladies (pourriture sèche, galle noire, chancre et flétrissement.) sur les tubercules de pomme de terre, causées par *Fusarium proliferatum, Rhizoctonia solani, Alternaria alternata* et *Wickerhamomyces anomalus* dans la région d'El Oued (Sahara septentrional Est algérien).

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Identification and Characterization of Some Phytopathogenic Fungi in Post-Harvest Potato (*Solanum tuberosum*) in the El Oued Region (Eastern Northern Sahara, Eastern Algeria)

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Abstract

The global staple crop, the potato (*Solanum tuberosum L.*), is susceptible to post-harvest rot caused by various fungal pathogens. These pathogens lead to a significant reduction in potato quality and marketable yield. Our study aimed to isolate and identify different phytopathogenic fungi present on potato tubers after harvest in the El Oued region of Eastern Sahara, Algeria. We utilized Potato's Dextrose Agar (PDA) culture medium for isolating and identifying molds. Macroscopic examination was performed with the naked eye and under a binocular microscope. Microscopic examination allowed us to determine certain morphological characteristics such as spore type and thallus shape. Total genomic DNA was extracted from seven-day-old cultures using a commercial NucleoSpin Plant II kit. Based on morphological features and molecular analyses of spore isolates, using nucleotide sequences from the internal transcribed spacer (ITS), the results of 18S rDNA analysis confirmed that the fungi infesting the tubers were identified as *Fusarium proliferatum*, *Alternaria alternaria*, *Rhizoctonia solani*, and *Wickerhamomyces anomalus*. This study represents the first report of *Fusarum proliferatum*, *Alternaria alternate*, *Rhizooctonia solani,* and *wickerhamommces anomalus* causing wilt diseases, dry rot, Canker and black scurf of potato tubers in southern Algeria.

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Contents

1 Introduction

The potato (*Solanum tuberosum L.*) is considered a staple vegetable for ensuring food security in developing countries (Haverkort & Struik, 2015). In terms of global consumption, it ranks first among non-cereal food crops. Water stress or low fertility can increase the susceptibility of this crop to certain diseases. High soil moisture favors the growth and spread of fungi, while wilting is more severe when soil moisture levels are low.

Fungal diseases play a central role in yield losses and are classified into foliar, soil-borne, and tuber diseases (Large, 1940). Late blight, early blight, and Phoma are among the foliar diseases, while common scab, black scurf, dry rot, and wilt are significant tuber diseases. Strategies for controlling fungal diseases primarily rely on the use of fungicides consisting of synthetic molecules. However, regardless of the recommended method for protecting crops against phytopathogenic agents, it is essential to identify them beforehand. In this context, this study aimed to isolate and identify different species of phytopathogenic fungi that attack potato tubers after harvest (Pedras & Ahiahonu, 2005; Martínez et al., 2017; Termorshuizen, 2007; Hammerschmidt, 1984).

2 Materials and Methods

Isolation and purification of fungi

In 2019 and 2022, potato tubers displaying symptoms such as brown spots, dark necrotic lesions, and sclerotia (Figure 1) were collected from potato plants in El Oued (Southeast Algeria). Microscopic examination of fragments from these infested tubers revealed the presence of spores, indicating a fungal infection. We used Potato's Dextrose Agar (PDA) culture medium, which is commonly recommended for mold research, enumeration, and maintenance of collected and sub-cultured strains (Botton et al., 1990). Small 5 mm diameter pieces of tubers were excised and sterilized in 70% ethanol for 30 seconds. They were then transferred to 1% NaOCl for 1 minute, rinsed with sterile distilled water, and inoculated onto Potato Dextrose Agar (PDA) plates. The plates were incubated at 25°C for 48 hours. After incubation, different fungal colonies were obtained. Each colony was isolated onto a new Petri dish to facilitate further examination. This step involves the purification of the isolated strains through a series of subcultures, which involve the aseptic transfer of the microorganism to a fresh and sterile medium to maintain it in pure culture (Botton et al., 1990).

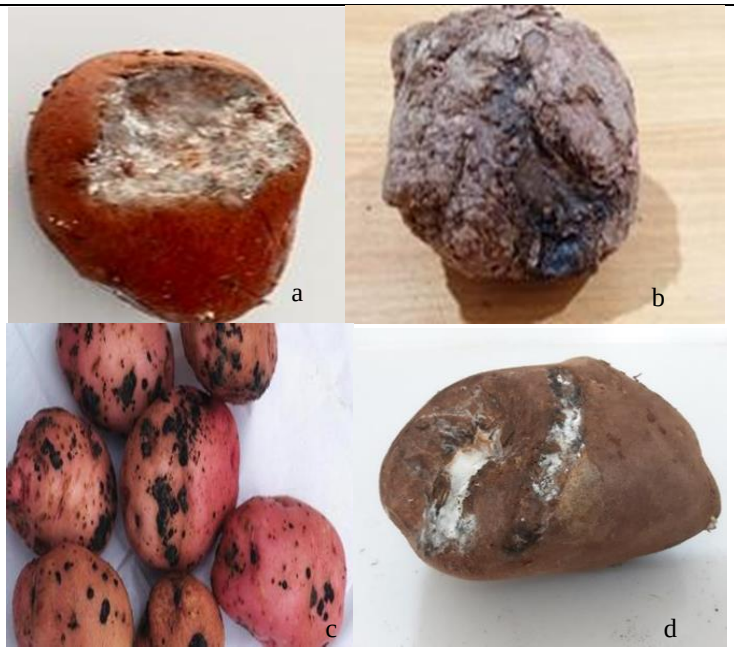

Figure 1. Symptoms of fungal diseases on potato tubers
a: Dry rot, b: Canker, c: Black scurf, d: Wilt

Morphological characterization of fungi

The purpose of identification is to classify fungal strains based on genus and species using identification criteria. It relies on two aspects: macroscopic and microscopic examination (Botton et al., 1990). Macroscopic examination of the Petri dishes was conducted with the naked eye and a binocular microscope. We carefully observed the external appearance of the fungi in a well-lit area, checked if all the colonies were identical, and noted their consistency (cottony, woolly, fluffy, powdery, etc.). Microscopic examination was based on morphological characteristics, including fruiting bodies, spore type, thallus shape, size, color, and spore arrangement (Bourgeois & Leveau, 1980).

DNA extraction, PCR amplification, and identification

Total genomic DNA was extracted from seven-day-old cultures using a commercial kit, NucleoSpin Plant II (Macherey-Nagel, Germany). The primers ITS1 CTTGGTCATT TAGAGGAA GTA A / ITS4 TCCTCCGCTTATTGATATGC (Gardes & Bruns, 1993) and EF2/EF-728F (Carbone & Kohn, 1999), were used for PCR amplification and sequencing of the translation elongation factor 1-alpha gene. The different steps involved in the process were as follows:

a) Initial denaturation for 5 minutes at 95°C, followed by a second denaturation step for 30 minutes (35 cycles) at 95°C.
b) Hybridization at 52-55°C for 30 minutes.
c) Extension at 72°C for 45 seconds.
d) Final extension at 72°C for 7 minutes.

The PCR reaction system consisted of 14.1 µl of ultra-pure water, 5 µl of Promega Taq Buffer (X5), 1.5 µl of 25 mM buffer (with MgCl2), 0.2 µl of dNTP (25 mM), 0.5 µl of forward primer (10 µM), 0.5 µl of reverse primer (10 µM), 0.2 µl of Promega Taq Polymerase (5 U), and 2 µl of genomic DNA. The amplification products were visualized after electrophoresis on a 1.5% agarose gel with the addition of 10 µl of PCR products. Migration

was followed by staining in an ethidium bromide bath (0.5 µg/ml). Subsequently, the DNA was visualized and photographed under UV light using the Bio-Rad Gel Doc system (USA).

The PCR products were purified using the NucleoSpin® Gel and PCR Clean-up kit from Macherey-Nagel (Germany). The isolated and purified PCR products were sequenced using the Sanger sequencing technique (Sanger et al., 1977), with the Applied Biosystems BigDye v3.1 kit.PCR primers were used to amplify the sequencing fragments. The obtained sequences were analyzed using the CHROMAS PRO software, and the final sequences were then compared to sequences in the GeneBank database using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) from NCBI. This comparison was done to identify the studied isolates based on the percentage of similarity with reference strains (Bharadwaj et al., 2008; Singh et al., 2022; van der Wolf & De Boer, 2007; Karnata & Putra, 2017).

3 Results and Discussions

Morphological and molecular identification/characterization of fungi

The colonies rapidly developed a white aerial mycelium that turned violet in the older culture. This is due to the production of a light violet pigment after 7 days on Potato Dextrose Agar (PDA) at 25°C (Figure 2) with a fluffy appearance covering the entire Petri dish. The results of the microscopic examination revealed that *F. proliferatum* had a large number of small microconidia without septa, measuring between 10 and 15 µm (Figure 2). These microconidia were abundant in the aerial mycelium and formed chains of variable length on both monophialides and polyphialides. These findings are consistent with those described by Leslie & Summerell (2008).

Figure 2. a- *Fusarium proliferatum* on PDA agar
b- *Fusarium proliferatum* under optical microscope at 40X magnification

Figure 3 displays a colony with colors ranging from olive green to black. The majority of colonies exhibit a fuzzy or cottony appearance and a notably slow growth rate on PDA medium. Microscopic observation of *A.alternata* colonies revealed significant sporulation, with numerous multicellular, greenish conidia surrounding the mycelium. Fungal identification was based on the morphological characteristics of the sporulation mode and conidia (Simmons, 1967).

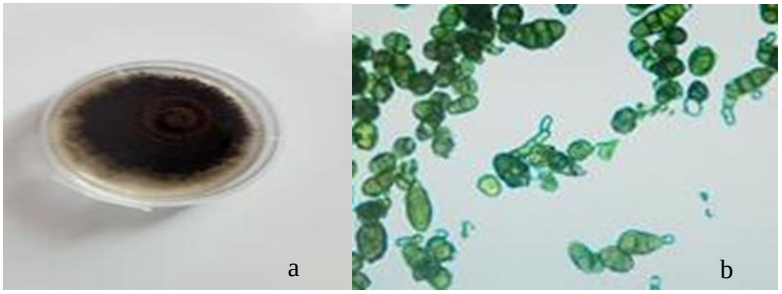

Figure 3. a- *Alternaria alternata* on PDA agar
b- *Alternaria alternata* under optical microscope at 40X magnification

The mycelial growth in Figure 4 exhibits a whitish appearance and rapidly covers the entire Petri dish. It gradually turns brown over time and displays dark brown sclerotia. Microscopic observation of pure colonies revealed the typical morphological characteristics of the *Rhizoctonia* genus, including colorless septate hyphae, a branching pattern at right angles, multinucleate hyphal cells, and hyphal constriction near their point of origin. These characteristics align with the description provided by Sneh et al. (2013).

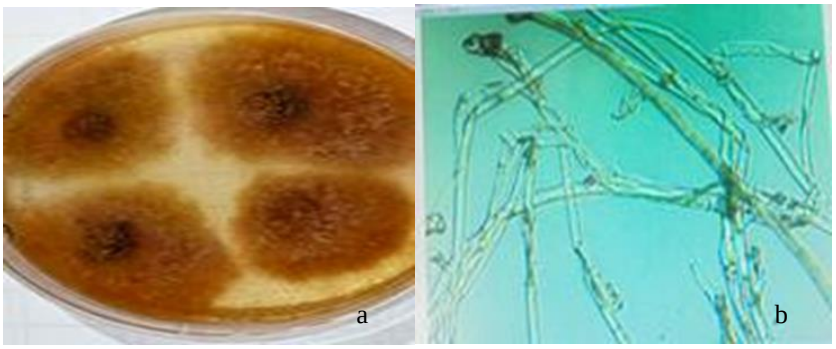

Figure 4. a- *Rhizoctonia solani* on PDA agar
b- *Rhizoctonia solani* under optical microscope at 40X magnification

The isolated white colonies in Figure 5 exhibited rapid cottony growth with a diameter of approximately 2 mm. Under the microscope, the cells appeared round, budding, dispersed, and reproductive. This description corresponds to that provided by Ma et al. (2021), for the *Wickerhamomyces* genus.

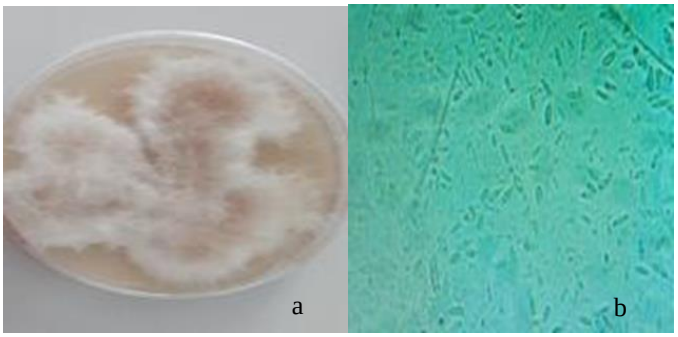

Figure 5. a- *Wickerhamomyces anomalus* on PDA agar
b- *Wickerhamomyces anomalus* under optical microscope at 40X magnification

The internal transcribed spacer (ITS) region of the fungal sequences was amplified by PCR and subjected to sequencing data in the GenBank NCBI (GenBank accession numbers OQ606246.1, OQ860003.1, OQ771178.1, and OQ606247.1). A BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the sequenced PCR products revealed that the isolated fungal strains were *Fusarium proliferatum* with a 99.77% identity, *Alternaria alternata* and *Rhizoctonia solani* with a 100% identity, and *Wickerhamomyces anomalus* with a 99.83% identity.

4 Conclusion

Fungal pathogens pose a significant threat and result in massive yield losses in potato cultivation, which is economically important both globally and in Algeria. In this study, we found that dry rot of potatoes was caused by *F. proliferatum*. It has been reported that this fungus causes rot in garlic roots (Leyronas et al., 2018), in France, Codonopsis (Gao et al., 2017), gerbera (Zhao et al., 2020), alfalfa (Cong et al., 2016) in China,

Aloe vera (Avasthi et al., 2018) in India, soybean (Díaz Arias et al., 2011) in the USA, and blueberry (Pérez et al., 2011) in Argentina. Other studies have revealed the presence of *Fusarium proliferatum* on apricot trees in Turkey (Ören et al., 2023), on Cyrtoneme polygonatum causing leaf blight in China (Zhou et al., 2021), on bananas in China (Huang et al., 2019), preserved garlic in Slovakia (Horáková et al., 2021), Syagrus romanzoffiana in Punjab-India (Faraz et al., 2020), chickpeas in Cuba (Duarte-Leal et al., 2020), and zucchinis in Morocco (Ezrari et al., 2020). *Alternaria* is an important fungal genus with a global distribution. This pathogenic Ascomycete can be found in plants as well as in humans (Thomma, 2003).

Studies have reported the presence of *Alternaria alternata* on peach trees in Pakistan (Alam et al., 2019), and Gerbera Jamesonii in Brazil (Bellé et al., 2019), *Xanthium strumarium L* (Abdessemed et al., 2019), in Algeria, pear trees (Chen et al., 2020), rose plants (Fang et al., 2020), and peanut crops (Zhang et al., 2021), in China. Garibaldi et al. (2023) mentioned its presence in Hydrangea paniculata in Italy, as well as in soybeans in Pakistan (Buzdar et al., 2023). Rhizoctonia, a potato disease, causes significant losses in marketable yield, which can reach up to 30% (Carling et al., 1989). Several studies have reported the presence of *Rhizoctonia solani*, such as on white cabbage in Turkey (Saygi et al., 2020), Campanula plants in Italy (Garibaldi et al., 2019), pepper plants in Kyrgyzstan (Erper et al., 2021), and sugar beet crops in Turkey (Avan et al., 2021).

Concerning *Wickerhamomyces anomalus*, few studies have been reported regarding this fungus to date. Only simple reports have been mentioned on the NCBI platform. Potatoes are typically harvested in February and March. Initially infected tubers in the fields can also develop rot during their storage in cold rooms. This results in significant losses for farmers as well as processing industries (Morrell & Rees, 1986; Fernandez-San Millan et al., 2021; Pedras et al., 2009; Suriani, 2019). The severity of the infection varies depending on the predominant species in a given area of potato cultivation. Species identification is the critical first step that provides a scientific basis for investigating the disease cycle, epidemiology, and management strategies of this important pathogen. To our knowledge, this is the first report confirming various diseases (dry rot, black scurf, canker, and wilting) on potato tubers caused by *Fusarium proliferatum, Rhizoctonia solani*, *Alternaria alternata*, and *Wickerhamomyces anomalus* in the El Oued region (Eastern Northern Sahara) of Algeria.

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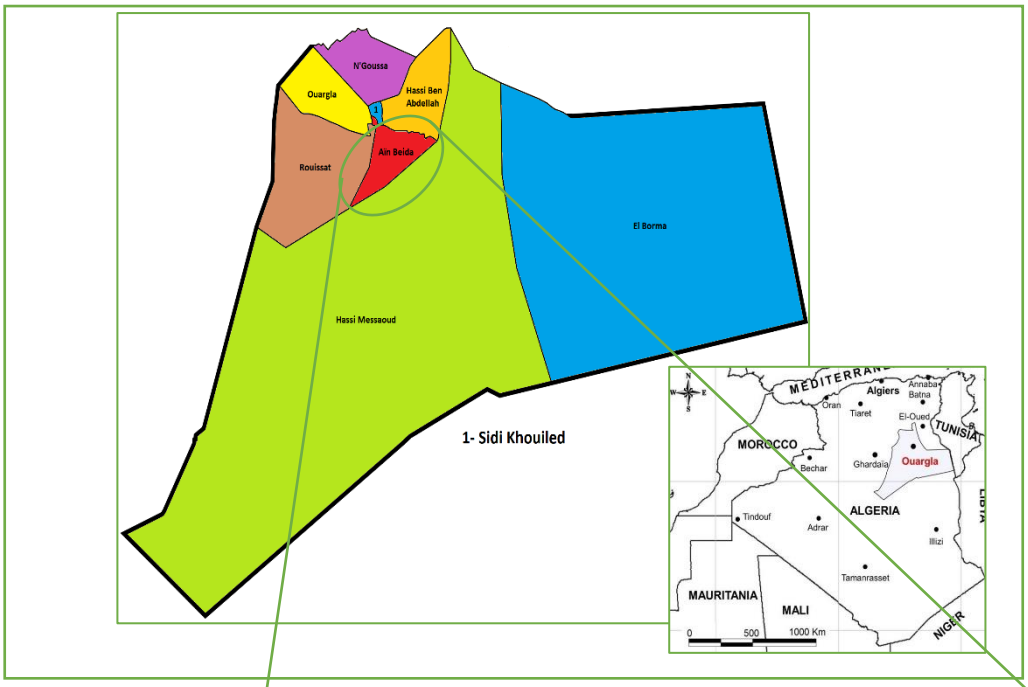
CHAPITRE II

Activité antifongique des huiles essentielles de quatre plantes médicinales sur des champignons isolés de tubercules de pomme de terre (*Solanum tuburosum*) de la région d'El Oued (Sahara septentrional Est Algérien)



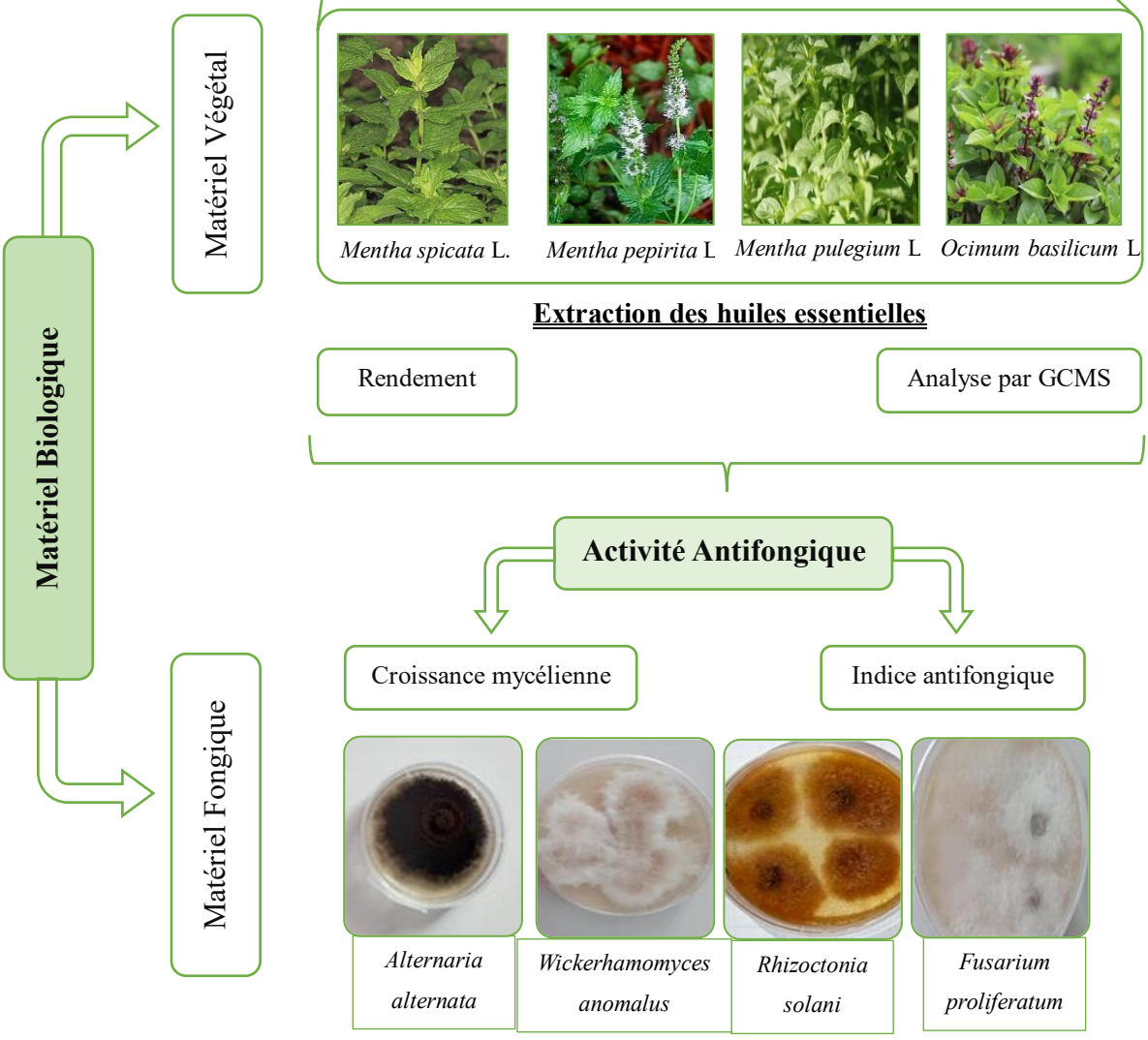


Figure 2 : Dispositif expérimental du Chapitre 2

Antifungal activity of essential oils of four medicinal plants on fungi isolated from potato tubers (*Solanum tuburosum*) from the El Oued region (Eastern Algerian Northern Sahara) (article Accepted: December 2023 dans la revue Tob Regul Sci)

Chapitre II : Activité antifongique des huiles essentielles de quatre plantes médicinales sur des champignons isolés de tubercules de pomme de terre (*Solanum tuburosum*) de la région d'El Oued (Sahara septentrional Est algérien).

Ce chapitre est consacré à l'étude de la composition chimique des huiles essentielles extraites des parties aériennes de 4 plantes : *Mentha spicata* L, *Mentha pepirita* L, *Mentha pulegium* L et *Ocimum basilicum* L, afin d'évaluer leurs propriétés antifongiques contre les 4 champignons pathogènes isolés et identifiés à partir de tubercules de pomme de terre en post récolte.

La méthodologie adoptée pour la réalisation de cette partie de travail est résumée dans la **Figure 2.**

Les plantes utilisées pour tester les vertus antifongiques de leurs huiles essentielles sont des plantes médicinales abondamment cultivées, à savoir la menthe verte (*Mentha spicata*), la menthe poivrée (*Mentha piperita*), la menthe pouliot (*Mentha pulegium*) et le basilic (*Ocimum basilicum*). Les agents pathogènes étudiés sont *Fusarium proliferatum, Alternaria alternata, Wickerhamomyces anomalus* et *Rhizoctonia solani*. Les souches phytopathogènes mises à l'essai dans le cadre de notre étude ont été purifiées puis identifiées par PCR dont le numéro d'accès GenBank NCBI (Centre national d'information sur la biotechnologie) est OQ606246.1, OQ860003.1, OQ771178.1 et OQ606247.1 **(photo 1, article).**

Les huiles essentielles ont été extraites par hydrodistillation puis analysées par chromatographie en phase gazeuse, couplée à la spectrométrie de masse (GS-MS) **(voir annexe III)** au niveau de laboratoire de recherche de Génie des Procédés, Université de Ouargla). Les résultats ont révélé que *M. pulegium* est la plus riche en huiles essentielles avec un rendement de 2.1%, suivie par *M. spicata* (1,4%), *M. piperita* (0,83%) et *O. basilicum* (0,19%) **(Tableau 1, article).** *Mentha spicata* est composée par (-)- Carvone 41.66, exo-2, 7,7-triméthylbicyclo [2.2.1] heptan- 10.28% et Isoborneol 5.83%. Ces résultats sont similaires à la plupart des travaux antérieurs.

Le (-)- Carvone constitue le composant principal de *M. piperita* (48,74%), s'en suivent le cis-dihydrocarvone (7,96 %) et le 2, 7,7-triméthylbicyclo [2.2.1] heptan-2-ol (18,84 %), confirmés par divers auteurs.

L'huile essentielle de *M. Pulegium* est caractérisée par la présence de (-)- carvone en tant que composant principal avec une teneur de 30,89%, d'isopelugone (23,62%), de cis-Dihydrocarvone 6.62%, et d'eucalyptol 5.78 Nos résultats diffèrent de la plupart des études réalisées par divers auteurs, tels que ceux de **Hmiri *et al* (2011)** au Maroc qui ont mentionné que le composant majoritaire était le R(+)-Pulègon à 80,28%, **Aissaoui *et al* (2018), Uwineza *et al*, (2018)** ont montré que le composant principal est la pulégone à des concentrations (67,63%) et (84,75%), respectivement. L'essence volatile *d'O. basilicum* contient du Linalol (26,16%), d'estragole (16,69%), de T-Cadinol (8,50%) et d'eucalyptol (4,72%). Nos résultats sont similaires à la majorité des travaux **(Tableau 2, article).**

L'activité antifongique des huiles essentielles a été évaluée par la méthode du contact direct contre la croissance mycélienne des souches testées. Les résultats ont indiqué un potentiel élevé d'activité antifongique vis-à-vis des souches testées. Les huiles essentielles de *Mentha spicata* **(Figure 5, article)** présentent un effet antifongique de 100% contre le *Fusarium proliferatum* à la concentration 0.90% et 0.95% pour *Alternaria alternata*, *Rhizoctonia solani* et *Wickerhamomyces anomalus*. Concernant les huiles essentielles de *Mentha pepirita*, **(Figure 6, article)** une inhibition totale (100%) de la croissance mycélienne a également été obtenue sur toutes les souches testées, aux concentrations 0.95% et 1%, et à 0.87% pour *Wickerhamomyces anomalus* et à 0.90% pour *A.alternata*. L'huile essentielle de *Mentha pulegium* **(Figure 7, article)** a un effet inhibiteur fongicide à la concentration 0,97% sur *Altenaria alternata* et *Fusarium proliferatum* ; à 0,95% sur *Wickerhamomyces anomalus* et *Rhizoctonia solani*. Concernant les huiles essentielles *d'Ocimum bacilicum*, **(Figure 8, article)** une inhibition totale (100%) de la croissance mycélienne des souches fongiques à la concentration 0.97% pour toutes les souches sauf *Wickerhamomyces anomalus* pour lequel l'effet fongicide a été obtenu à la concentration 1%.

Les huiles essentielles testées montrent clairement des effets anti-croissance sur les champignons étudiés. Elles peuvent être utilisées comme alternatives aux fongicides synthétiques dans les stratégies de contrôle des agents pathogènes, responsables de la pourriture des tubercules de pomme de terre tout en protégeant l'environnement.

Antifungal activity of essential oils of four medicinal plants on fungi isolated from potato tubers (*Solanum tuburosum*) from the El Oued region (Eastern Algerian Northern Sahara)

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Abstract

The aim of this study is to investigate the chemical composition of essential oils extracted from the aerial parts of four plants: *Mentha spicata L, Mentha pepirita L, Mentha pulegium L*, and *Ocimum basilicumL*, in order to evaluate their antifungal properties against several pathogenic fungi isolated from post-harvest potato tubers.The essential oils were extracted through hydrodistillation and analyzed using gas chromatography-mass spectrometry (GC-MS)The results revealed that *M. pulegium* has the highest content of essential oils with a yield of 2.1%, followed by *M. spicata* (1.4%), *M. piperita* (0.83%), and *O. basilicum* (0.19%).*M.spicata* is composed of (-)-Carvone (41.66%) and exo-2,7,7-trimethylbicyclo[2.2.1]heptane (10.28%).(-)-Carvone is the main component of *M.piperita* (48.74%), followed by cis-dihydrocarvone (7.96%) .The essential oil of *M. pulegium* is characterized by the presence of (-)-carvone as the main component with a content of 30.89%, isopelugone (23.62%).The volatile essence of *O. basilicum* contains linalool (26.16%), estragole (16.69%).The antifungal activity of the essential oils was evaluated using the direct contact method against the mycelial growth of the tested strains. The results indicated a high potential for antifungal activity against the tested strains.The essential oils of *Mentha spicata* showed a 100% antifungal effect against *Fusarium proliferatum* at a concentration of 0.90% and 0.95% for *Alternaria alternata*,*Rhizoctonia solani*,and *Wickerhamomyces anomalus*.Regarding the essential oils of *Mentha piperita*, a complete inhibition (100%) of mycelial growth was also achieved against all tested strains, at concentrations of 0.95% and 1%, and at 0.87% for *Wickerhamomyces anomalus* and 0.90% for *A. alternata*. The essential oil of *Mentha pulegium* has

a fungicidal inhibitory effect at a concentration of 0.97% on *Alternaria alternata* and *Fusarium proliferatum*, and at 0.95% on *Wickerhamomyces anomalus* and *Rhizoctonia solani*. Regarding the essential oils of *Ocimum basilicum*, a complete inhibition (100%) of mycelial growth of fungal strains was achieved at a concentration of 0.97% for all strains except *Wickerhamomyces anomalus*, for which the fungicidal effect was obtained at a concentration of 1% .The obtained results, pave the way for the utilization of essential oils as an alternative to chemical fungicides.

Keywords: : Potato, medicinal plants, phytopathogenic fungi, essential oils, antifungal activity.

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1. Introduction

The potato (*Solanum tuberosum L.*) is one of the most important crops worldwide and ranks fourth among all food crops in total production **(FAO, 2022).** However, potatoes are susceptible to certain diseases caused by various fungal pathogens, such as *Colletotrichum coccodes***(Johnson *et al.,* 2018)**, which causes black dot disease, and *Helminthosporium solani Dur*, which causes silver scurf **(Massana-Codina *et al.,* 2021).**

These pathogens result in significant post-harvest losses. To address these issues, chemicals are commonly used, but they come with limitations and numerous drawbacks, such as pollution, phytotoxicity, disruption of biological balance, and particularly the risk of developing fungicide-resistant strains **(Arias-Rivas *et al.*, 1998; Dorrance *et al.,* 2004).**

The challenges associated with synthetic fungicides have led to the search for more effective and environmentally friendly alternative solutions **(Wilson *et al.,* 1993).** Several studies have been conducted to explore the potential of essential oils against phytopathogenic fungi **(Neri *et al.*, 2006; Amiri *et al.*, 2008)**. Due to their natural origin, these oils contribute to the safety of humans and the environment. Furthermore, they present a low risk of development of resistance by pathogenic microorganisms **(Tatsadjieu *et al.*, 2010).** The purpose of this study is to investigate the antifungal properties of essential oils from four medicinal plants against post-harvest phytopathogenic fungi of potatoes.

I-Methods and Materials

I-1-Plant Material

The plants used to test the antifungal properties of their essential oils are widely cultivated medicinal plants family Lamiaceae, namely spearmint (*Mentha spicata*), peppermint (*Mentha piperita*), pennyroyal (*Menthapulegium*), and basil (*Ocimum basilicum*). Their aerial parts were harvested in August 2020, in the Ouargla region (Southeast Algeria) coordinates (N31°57'47" E 5°20'31"), and then dried in the shade for 15 days.

I-2-Fungal Material

The fungi used in this study were isolated from potato tubers showing characteristic symptoms of fungal attack, obtained during post-harvest from the El Oued province coordinates (N33°07',

7°11' E). Four species were selected: *Fusarium proliferatum*, *Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani*. The phytopathogenic strains tested in our study were purified and identified using PCR, with the GenBank NCBI (National Center for Biotechnology Information) accession numbers OQ606246.1, OQ860003.1, OQ771178.1, and OQ606247.

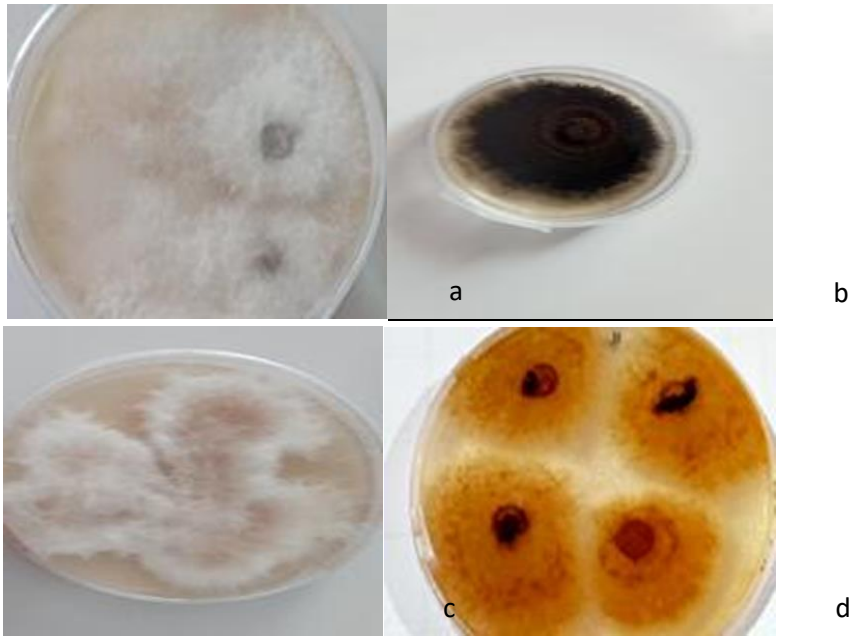

Photo 01: Phytopathogenic fungi on PDA agar.

a: *Fusarium proliferatum*, **b**: *Alternaria alternata*, **c:***Wickerhamomyces anomalus*, **d:***Rhizoctonia solani*.

I-3-Extraction of Essential Oils

The extraction of essential oils was performed by hydrodistillation using a Clevenger-type apparatus. Approximately 100 g of dried aerial parts of plants were immersed in a 1000 ml flask containing a sufficient volume of water and subjected to extraction for 3 hours. The resulting oil was stored in the dark at 4°C (**Kizil *et al.*, 2010**).

I-4-Determination of the Chemical Composition of Essential Oils by GC/MS

The chromatographic analysis of the essential oil was performed using a Bruker SCION 436 GC gas chromatography system coupled to a mass spectrometer (GC/MS). Electron impact fragmentation at 70 eV was used. The column used was an HP-5MS capillary column (15m x 0.25mm) with a film thickness of 0.25µm. The stationary phase of the column consisted of 5% phenyl and 95% dimethylpolysiloxane. The operating conditions were as follows: injector temperature of 250°C in a split mode of 1:50, temperature programming from 70°C to 280°C at a rate of 10°C/min, and the carrier gas used was helium with a flow rate of 1.5 ml/min. The temperatures of the quadrupole source were set at 250°C and 220°C, respectively. The linear retention indices (kI) for all compounds were determined using n-alkanes C10-C40. The identification of different constituents was carried out by comparing their mass spectra with those of reference products in available computerized libraries (NIST and Wiley).

I-5-Antifungal Activity

I-5-1-Direct Contact Methods

According to **Mohammedi *et al.,* 2012**, to evaluate the antifungal activity of the essential oils, we used the direct contact technique. This involved adding the oil at various concentrations to the liquid PDA culture medium at a temperature of 25°C, followed by thorough mixing for 2 minutes to ensure homogeneity. After agitation, the mixture (PDA + EO + a few drops of Tween 20) was poured into 50mm Petri dishes. Once solidified, inoculation was performed under a hood by placing a 5mm diameter mycelial disk obtained from a young culture (7 days of incubation) at the center of each Petri dish.

Control experiments were conducted under the same conditions without essential oil. The Petri dishes (both control and test samples) were incubated in a 25°C ± 2°C environmental chamber. Three replicates per treatment were performed to assess the antifungal effect of the tested oils. The radial mycelial growth was measured daily for a 10-day incubation period. The following parameters were evaluated:

I-5-2-Mycelial Growth

Mycelial growth (expressed in mm) was evaluated at the end of the experiment, after 10 days of incubation (240 hours), by measuring the average of three perpendicular diameters passing through the center of the mycelial disk. This measurement was always compared to the control cultures that started on the same day and under the same conditions.

I-5-3-Determination of Antifungal Index

For each treatment, the antifungal index, expressed as a percentage, is calculated by the reduction in mycelial diameter growth compared to the control, using the following formula:

$I\ (\%) = [1 - (D_{test} / Dc_{ontrol})] \times 100$ (**Kordali *et al.,* 2003**).

$D_{control}$: Mycelial diameter growth in a medium without the presence of the essential oil (control).

D_{test}: Mycelial diameter growth in the presence of the essential oil (test).

I-6- Statistical Analysis

Based on the obtained results for each parameter, we calculated the means, standard deviations, and conducted an analysis of variance (ANOVA) using XLSTAT software (2019).

II/ Results and Discussion

II-1- Essential Oil Extraction Yields

The essential oil extraction yields (w/w, %), obtained after a 3-hour period of hydrodistillation of the tested plant species, are presented in **table 01.**

Table 01: Essential oil yield of the plants studied

	Mentha spicata	*Mentha piperita*	*Mentha pulegium*	*Ocimum basilicum*

Yields (%)	1.4	0.83	2.1	0.19

The data analysis from the above table reveals that the essential oil yields vary among the plant species and range from 0.19 to 2.1%. *M. Pulegium* exhibited the highest essential oil content with a yield of 2.1%, followed by *M.spicata* (1.4%), *M. piperita* (0.83%), and *O. basilicum* (0.19%).

The yield of *M. spicata* is higher than the one reported by **Dib *et al.* (2013)** which mentioned 1.27%. Similarly, the yield of *M. piperita* is higher than the one reported by **Likibi *et al.* (2015)** which corresponds to 0.52%. Our results regarding *M. pulegium* (1.9%) are close to those obtained by **Uwineza (2018)** in Morocco. However, the yield of essential oil in *O. basilicum* is lower than 0.44% reported by **Akono *et al.* (2012)** and 0.63% by **Kpodekon *et al.* (2013).** These observed differences in yield could be attributed to the collection area, soil properties, plant developmental stage, and the organs and extraction methods used.

II-2- Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of the Essential Oil.

The chemical composition of the studied plant's essential oils was determined using gas chromatography coupled with mass spectrometry (GC-MS). The identification of the chemical composition of the essential oils **(Table 02)** revealed a number of compounds, representing 82.58% of the total composition of *M. spicata*, 92.19% of *M. piperita*, and 89.52% of *M. pulegium*. The total chemical composition percentage of *O.basilicum* essential oil is 86.41%.

Table 02: Chemical composition of the essential oils of the studied plants.

Compounds	kIexp	kIlit	*Mentha spicata*	*Mentha pepirita*	*Mentha pulegium*	*Ocimum basilicum*
5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	915	924	0.03	0.27	_	_
α-Pinene	930	939	0.33	1.28	_	_
Camphene	938	946	0.16	0.31	0.49	_
(-)-Sabinene	962	967	0.86	_	_	_
β-Pinene	974	979	2.8	**6.43**	1.32	0.42
Myrcene	981	990	_	_	0.62	0.33
exo-2,7,7-trimethylbicyclo[2.2.1]heptan-	1124	1170	10.28	18.84	_	_

Eucalyptol	1026	1031	_	_	**5.78**	**4.72**
β –Ocimene	1044	1044	0.16	0.13	_	_
3-Carene	1008	1008	_	0.11	0.02	_
Terpinolen	1181	1188	0.08	0.09	_	_
Neo-dihydrocarveol	1180	1190	_	_	0.36	_
Linalool	1085	1100	_	0.54	_	**26.16**
(-)-dihydrocarveol	1119	1119	_	_	0.15	_
(+)-(E)-Limonene oxide	1122	1140	0.28	0.10	_	_
Myroxide	1140	1160	_	_	_	0.17
L-Camphor	1141	1143	_	_	_	0.64
cis-Verbenol	1144	1144	_	_	0.45	_
L-Menthone	1148	1150	_	_	2.21	0.15
Isoborneol	1149	1158	**5.83**	2.27	0.14	0.39
cis-Dihydrocarvone	1184	1190	**4.71**	7.96	**6.62**	_
L-**α**-Terpineol	1186	1186	2.23	_	3.08	_
Estragole	1190	1208	_	_	_	**16.69**
Isopulegone	1208	1208	_	_	**23.62**	1.70
Fenchyl acetate	1223	1232	_	_	_	0.18
D-Carvone	1243	1239	_	_	_	0.89
Chavicol	1247	1250	_	_	_	2.38
cis-Carvotanacetol	1248	1244	_	_	0.10	_
Citronellyl formate	1250	1260	_	_	0.31	_
(-)-Carvone	1258	1243	**41.66**	**48.74**	**30.89**	_
Bornyl acetate	1285	1270	_	0.13	_	1.42

Geranyl formate	1298	1300	_	_	0.22	_
Dihydrocarvyl acetate	1301	1303	0.04	0.19	_	_
α –Cubebene	1342	1348	_	_	_	0.12
Piperitenone	1344	1343	_	0.33	2.58	_
Phenol, 2-methoxy-3-(2-propenyl)-	1360	1360	0.08	_	_	4.33
cis-Carvyl acetate	1362	1362	0.10	0.12	_	_
Jasmone	1370	1380	_	_	0.07	_
α-Copaene	1388	1388	_	_	_	0.34
β -Bourbonene	1388	1389	1.28	_	0.94	0.46
(-)- β -elemene	1389	1390	_	0.09	0.10	2.09
trans-α -Bergamotene	1410	1411	_	_	_	2.88
Geranylacetone	1420	1430	0.19	0.07	0.23	_
β -farnesene	1448	1442	_	_	_	0.77
Humulene	1449	1454	0.92	_	0.87	_
cis-Muurola-4(15),5-diene	1460	1466	0.43	0.20	0.23	0.74
Germacrene D	1478	1485	0.21	0.10	0.10	_
α -Bulnesene	1490	1505	_	_	_	0.99
Germacrene A	1500	1508	0.03	_	_	_
(-)-gamma-cadinene	1504	1514	0.89	_	0.31	_
Calamenene	1517	1522	0.47	0.21	0.22	0.58
Sesquisabinene	1519	1534	_	_	_	0.18
Cubebol	1530	1535	_	_	_	0.33
Nerolidol	1558	1560	_	_	_	1.02

(-)-Spathulenol	1570	1577	_	_	_	3.11
Caryophyllene oxide	1575	1583	1.70	0.54	2.40	_
(-)-Globulol	1576	1590	_	_	_	0.34
Humulene epoxide II	1599	1597	0.20	_	0.05	_
Epicubenol	1606	1612	0.98	0.38	0.84	2.15
T-Cadinol	1613	1638	4.41	2.41	3.14	8.50
α-Cadinol	1641	1652	0.72	0.27	0.48	_
4(15),5,10(14)-Germacratrien-1-ol	1670	1681	0.10	_	_	_
Caryophylladienol II	1672	1678	0.22	_	0.29	_
Bisabolol	1675	1685	0.20	0.08	0.29	0.92
β -Sinensal	1678	1699	_	_	_	0.13
Aromadendrane	1679	1678	_	_	_	0.19
Total %			82.58	92.19	89.52	86.41
Hydrocarbon monoterpenes			4.42%	8.62%	2.45%	0.75%
Oxygenated monoterpenes			65.07%	78.78%	75.98%	55.84%
Hydrocarbon sesquiterpenes			4.23%	0.6%	2.77%	8.57%
Oxygenated sesquiterpenes			8.53%	3.68%	7.49%	16.69%
Others			0.33%	0.51%	0.83%	4.56

kI exp: Experimental retention index. **kI** lit: Literature retention index.

* The values in bold indicate the percentages of major compounds.

These results indicate that the essential oil of *M.spicata***(Table 02)** is primarily composed of monoterpenes (69.49%), with a predominance of oxygenated compounds (65.07%), while hydrocarbon monoterpenes constitute a minor fraction (4.42%). Sesquiterpenes account for 12.76% of the composition, with a prevalence of oxygenated compounds (8.53%) and hydrocarbon sesquiterpenes (4.23%). The essential oil of *M. piperita* is also predominantly monoterpenic (87.4%), with a higher proportion of oxygenated monoterpenes (78.78%) compared to hydrocarbon monoterpenes (8.62%). Sesquiterpenes represent 4.28% of the composition, with a prevalence of oxygenated compounds (3.68%) and hydrocarbon sesquiterpenes (0.6%). The essential oil of *M. pulegium* is primarily composed of monoterpenes (78.43%), with a predominance of oxygenated monoterpenes (75.98%), while hydrocarbon monoterpenes constitute a minor fraction (2.45%). Within the sesquiterpene group (10.26%), oxygenated compounds account for 7.49%, while hydrocarbon sesquiterpenes occupy a proportion of 2.77%. The essential oil of *O.basilicum* is predominantly monoterpenic (56.59%), with the majority composed of oxygenated monoterpenes (55.84%), while hydrocarbon monoterpenes are in the minority (0.75%). Sesquiterpenes represent 25.26% of the composition, with 16.69% being oxygenated compounds and 8.57% being hydrocarbon compounds.

The essential oils of *M. spicata*, *M. piperita* and *M. pulegium* are mainly composed of Carvone, with respective values of 41.66%, 48.74%, and 30.89% (**Table 02**). The main constituent of *O. basilicum* oil is Linalool (26.16%).

Mentha spicata is composed of (-)-Carvone (41.66%), exo-2,7,7-trimethylbicyclo[2.2.1]heptane (10.28%), isoborneol (5.83%), cis-Dihydrocarvone (4.71%), T.-Cadinol (4.41%), β-Pinene (2.8%), L-α terpineol (2.23%), caryophyllene oxide (1.70%), and β-Bourbonene (1.28%), totaling 85.41%. These results are consistent with most of the previous studies conducted. In Algeria, the essential oil of *Mentha spicata* is predominantly composed of carvone (59.40%), limonene (6.12%), and 1,8-cineole (3.80%) (**Boukhubti *et al.,* 2011**). In Morocco, carvone (29%) and trans-carveol (14%) were predominant (**Znini *et al.*, 2011**). In Tunisia, carvone (40.8%) and limonene (20.8%) were found to be major constituents **(Snoussi *et al.,* 2015),** while in Iran, carvone constituted 78.78% and limonene accounted for 11.50% (**Shahbazi *et al.,* 2015).**

Mentha piperita is primarily composed of (-)-Carvone (48.47%), accompanied by other constituents in relatively low amounts: 2,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol (18.84%), cis-Dihydrocarvone (7.96%), beta-Pinene (6.43%), T.-Cadinol (2.41%), and Isoborneol (2.27%), α-Pinene (1.28%), totaling approximately 92.77%. When comparing the chemical composition of *Mentha piperita* essential oils from different geographical origins worldwide, we observe that the oil from Algerian origin is similar to the one studied in our research.

The essential oil of *Mentha piperita* is predominantly composed of Carvone (51.04%), β-Pinene (1.66%), Caryophyllene (0.37%), and α-Pinene (1.07%) in Algerian oil **(Goudjil *et al.,* 2016),** while in Brazilian essential oil, Carvone is also the major compound, but with a percentage of 30.5% **(Gracindo *et al.,* 2006).** However, the essential oil differs from the one from Congo, where the major constituents are Methyl acetate (36.96%), Menthol (41.81%), and L-Menthone (5.12%) **(Likibi *et al.,* 2015).**

Indeed, it differs from the essential oil of Serbia, which is composed of menthol (37.4%), Methyl acetate (17.4%), and menthone (12.7%) **(Sokovic *et al.,* 2009),** as well as the essential oil of Turkish origin, which consists of (+)-menthol (38.06%), menthol (35.64%), and neo-menthol (6.73%) **(Kizil *et al.,* 2010).** The essential oil from Iran contains menthofuran (11.18%) and 1,8-cineole (6.69%) **(Mohammed *et al.,* 2012).** The essential oil from Morocco is dominated by menthone (29.01%), followed by menthol (5.58%), and Methyl acetate (3.34%) **(Derwich *et al.*, 2010).** The chemical composition of the Egyptian essential oil **(Gharib and Teixeira da Silva,2012)** differs from our oil due to the presence of 1,8-cineole (8.69%) as well as a high content of neo-menthol (40.47%).

The essential oil of *Mentha pulegium* is characterized by the presence of (-)-carvone as the main component, accounting for 30.89% of the oil composition. Other significant constituents include isopelugone (23.62%), cis-Dihydrocarvone (6.62%), eucalyptol (5.78%), T.-Cadinol (3.14%), L-α-Terpineol (3.08%), Piperitenone (2.58%), Caryophyllene oxide (2.40%), L-Menthone (2.21%), and β-Pinene (1.32%), totaling 90.03%. These results differ from most studies conducted by various authors. For example, **Hmiri *et al.* (2011)** in Morocco reported that the major component was R(+)-Pulegone at 80.28%. **Aissaoui *et al.* (2018)** and **Uwineza *et al.* (2018)** showed that Pulegone was the principal component at concentrations of 67.63% and 84.75%, respectively. Additionally, studies conducted in Tunisia by **Snoussi *et al.* (2008)** and **Hajlaoui *et al.* (2009)** revealed that Pulegone was the main compound in *Mentha pulegium*, with percentages of 44.27% and 61.11%, respectively. In Algeria, the major components of the essential oil are Pulegone (38.81%), Menthone (19.24%), and Piperitenone (16.52%) **(Boukhubti *et al.*, 2011).**

The volatile essence of *O. basilicum* obtained in our results contains linalool (26.16%) as the major compound, followed by estragole (16.69%), T-Cadinol (8.50%), eucalyptol (4.72%), 2-methoxy-3-(2-propenyl)-4.33 phenol,(-)-spathulenol (3.11%), trans-α-bergamotene (2.88%), chavicol (2.38%), epicubenol (2.15%), (-)-β-elemene (2.09%), isopulegone (1.70%), bornyl acetate (1.42%), and nerolidol (1.02%), totaling 87.38%. %. Our results are similar to the majority of studies, such as in Australia where linalool (28.6%), methyl chavicol (21.7), (E)-methyl cinnamate (14.3), α-cadinol (7.1), eugenol (5.9), and 1,8-cineole (4.0) were identified **(Politeo *et al.*, 2007)**, in Bulgaria where linalool (54.95%), methyl chavicol (11.98%), and methyl cinnamate (7.24%) were found **(Opalchenova, 2003),** and in the USA where linalool

(3.94%), methyl chavicol (2.03%), and methyl cinnamate (1.28%) were present **(Lee, 2005).** However, the essential oil of *Ocimum basilicum* from Benin presents different major compounds, including estragole (31.33%), linalool (24%), and eugenol (21.67%) (**Kpodekon *et al.,* 2013).** In Cameroon, **Akono *et al.* (2012)** reported that the essential oil of *Ocimum basilicum* contains limonene (30.9%) as the major compound, followed by linalool (18.9%). In Brazil, the major constituents are linalool (72.14%), geraniol (12.95%), and 1,8-cineole (7.90%) **(Oliveira, 2009).** In Poland, the detected compounds include linalool (64.7%), geraniol (12.6%), 1,8-cineole (4.1%), and epi-α-cadinol (3.8%) (**Nurzyn´ska-Wierdak, 2013).** In Oman, linalool is also the predominant component (69.9%), followed by geraniol (10.9%), 1,8-cineole (6.4%), α-bergamotene (1.6%), and geranyl acetate (1.4%) **(Hanif, 2011).**

Generally, the variation in the chemical composition of essential oils from the same species can be attributed to geographical origin, extraction technique, harvest time, and climatic factors.

II-3- Antifungal Activity

II-3-1- Mycelial Growth

The antifungal activity is determined by the absence or presence of mycelial growth. The analysis of variance indicates a highly significant difference ($p<0.001$) for the mycelial growth of the tested fungal strains.

Figure 01 represents the fungal species growth results obtained using *Mentha spicata* essential oil. All fungal strains showed hyphal development at concentrations of 0.80% and 0.85%. Mycelial growth slightly decreases with increasing essential oil concentration compared to the control, with a diameter of 11.6mm for *Wickerhamomyces anomalus* at a concentration of 0.90% and 9mm for *Rhizoctonia solani.* For *Alternaria alternata*, a diameter of 5.4mm was recorded at a concentration of 0.88%, but no growth (0mm) was observed for *Fusarium proliferatum* at 0.90%. No mycelial growth was obtained at essential oil concentrations of 0.90% and 1%. The essential oils also exhibit inhibitory effects on the fungi, with minimum inhibitory concentrations (MIC) of 0.90% for *F. proliferatum* and *A. alternata,* and 0.95% for *W. anomalus* and *R. solani.*

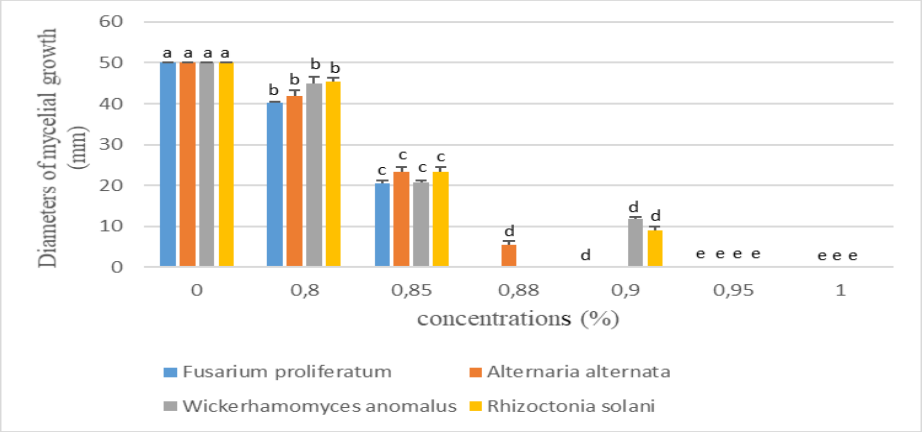

Figure 01: Effect of different concentrations of *Mentha spicata* essential oils on mycelial growth.

Figure 02 shows that Mentha piperita essential oil inhibits the growth of *Fusarium proliferatum*, *Alternariaalternata,Wickerhamomyces anomalus*, and *Rhizoctonia solani*. No mycelial growth was observed at 0.95% and 1% concentrations. At 0.90% concentration, a mycelial growth diameter of 14.2 mm was recorded for *Fusarium proliferatum* and 10.2 mm for *Rhizoctonia solani*, but no growth (0 mm) was observed for *Alternaria alternata* and *Wickerhamomyces anomalus*. At 0.85% concentration, a mycelial growth diameter of 9 mm was noted for *Wickerhamomyces anomalus*, and 0 mm at 0.87%. For *Fusarium proliferatum*, a diameter of 34.4 mm was recorded, for *Alternaria alternata* (20.2 mm), and for *Rhizoctonia solani* (20 mm). The MIC was determined to be 0.95% for *Fusarium proliferatum* and *Rhizoctonia solani*, 0.90% for *Alternaria alternata*, and 0.87% for *Wickerhamomyces anomalus*. The largest mycelial growth diameter (50 mm) was recorded in the control.

The analysis of variance indicates a highly significant difference ($p<0.001$) for the mycelial growth of the tested fungal strains.

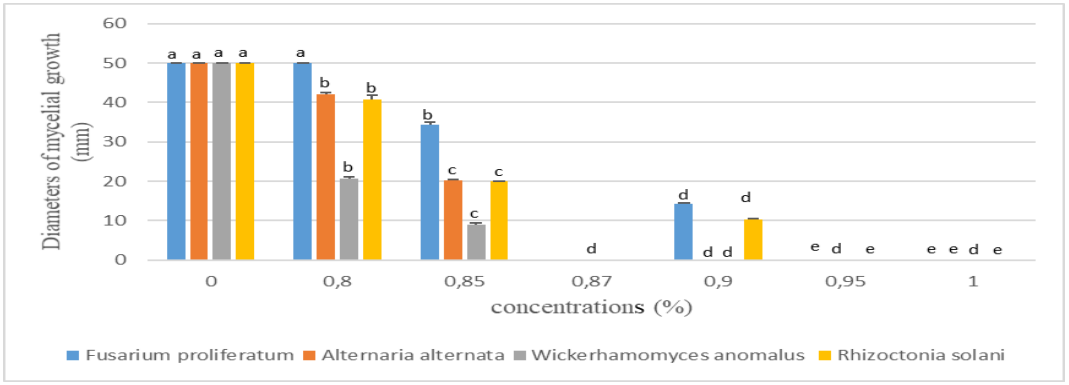

Figure 02: Effect of different concentrations of *Mentha piperita* essential oils on mycelial growth.

Figure 03 shows the results of the effects of *Mentha pulegium* on the growth of fungal species. The analysis of variance reveals a highly significant difference ($p<0.001$) for the mycelial growth of the tested fungal strains.

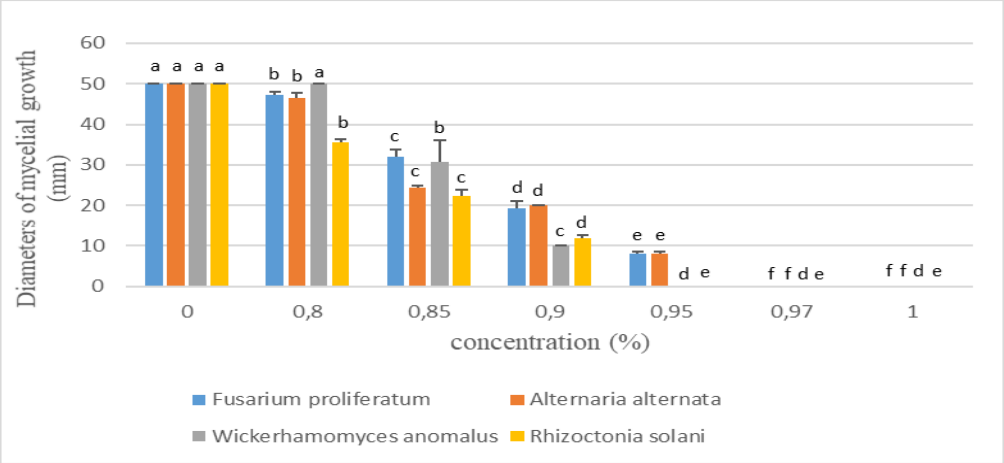

Figure 03: Effect of different concentrations of *Mentha pulegium* essential oils on mycelial growth.

A maximum mycelial growth diameter (50 mm) of *Fusarium proliferatum*, *Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani* was obtained in the control group, corresponding to the absence of essential oils. Mycelial growth was observed for all strains at concentrations of 0.80%, 0.85%, 0.90%, and 0.95% in the presence of the essential oil of *Mentha pulegium*, except for *Wickerhamomyces anomalus* and *Rhizoctonia solani* at a concentration of 0.95% (0 mm). Mycelial growth slightly decreased with increasing essential oil concentration compared to the control. At concentrations of 0.97% and 1%, a diameter of 0 mm was recorded for all strains. The minimum inhibitory concentration (MIC) was determined to be 0.97% for *Fusarium proliferatum* and *Alternaria alternata,* and 0.95% for *Wickerhamomyces anomalus* and *Rhizoctonia solani*.

The analysis of variance indicates a highly significant difference ($p<0.001$) for the mycelial growth of the tested fungal strains. According to Figure 04, the effect of *Ocimum basilicum* essential oil was only observed at concentrations of 0.97% and 1%, where no growth was obtained for all the fungi. Mycelial growth slightly decreased with increasing essential oil concentration compared to the control, with a diameter of 4.2 mm for *Fusarium proliferatum*, 8.2 mm for *Alternaria alternata*, and 10 mm for *Wickerhamomyces anomalus* at a concentration of 0.95%. However, for *Rhizoctonia solani*, it reached 4 mm. In the control group, all four strains exhibited growth, reaching a diameter of 50 mm. The minimum inhibitory concentration (MIC) was determined to be 0.97% for *Fusarium proliferatum*, *Alternaria alternata*, and *Rhizoctonia solani*, and 1% for *Wickerhamomyces anomalus*.

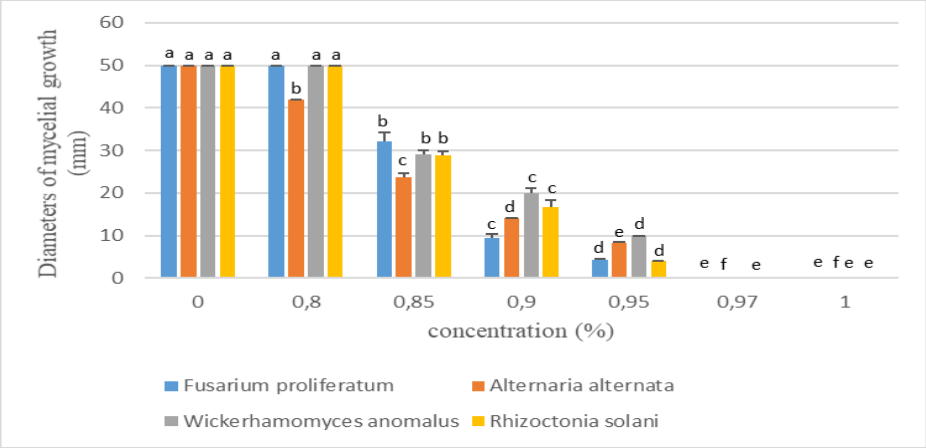

Figure 4: Effect of different concentrations of *Ocimum basilicum* essential oil on mycelial growth.

II-3-2. Determination of antifungal index

Regarding *Mentha spicata*, **Figure 5** also shows 100% inhibition of mycelial growth for *Fusarium proliferatum,Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani* at concentrations of 1% and 0.95%, as well as at 0.90% for *Fusarium proliferatum*. We obtained 19.6% inhibition at the concentration of 0.80% for *Fusarium proliferatum,* 16.4% for *Alternaria alternata*, 10% for *Wickerhamomyces anomalus*, and 9.2% for *Rhizoctonia solani*. The antifungal index increases as the concentration of the essential oil increases, reaching 89% at the concentration of 0.88% for *Alternaria alternata*.

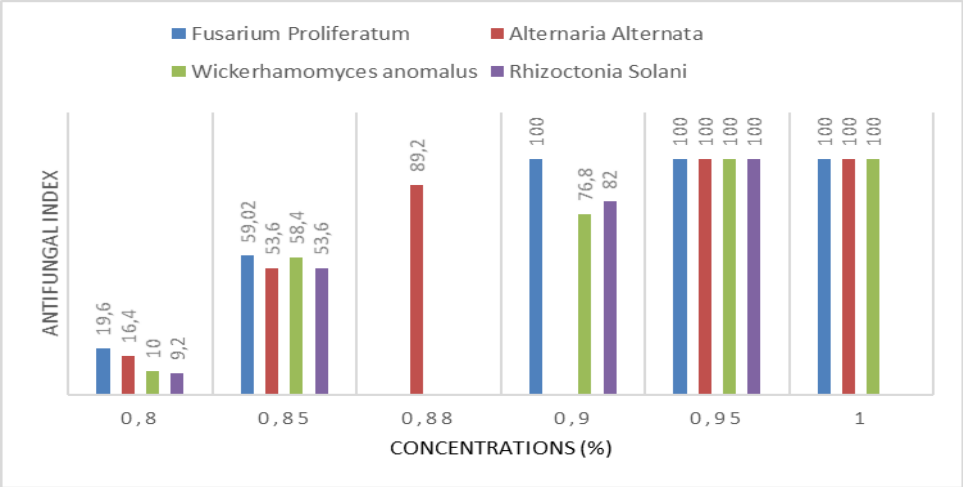

Figure 5: Antifungal index of inhibition of mycelial growth for different concentrations of *Mentha spicata* essential oil.

Figure 6 also shows a complete inhibition (100%) of mycelial growth for all tested strains at concentrations of 1% and 0.95%. At 0.90%, effectiveness is observed for *Fusarium proliferatum*, *Alternaria alternata*, and *Wickerhamomyces anomalus*, and at 0.87% for *Wickerhamomyces*

anomalus. However, we obtained 0% inhibition at the concentration of 0.80% for *Fusarium proliferatum*, 15.6% for *Alternaria alternata*, 58.8% for *Wickerhamomycesanomalus,* and 18.4% for *Rhizoctonia solani* of essential oil. The antifungal index increases as the concentration of the essential oil is increased, reaching 0.85%.

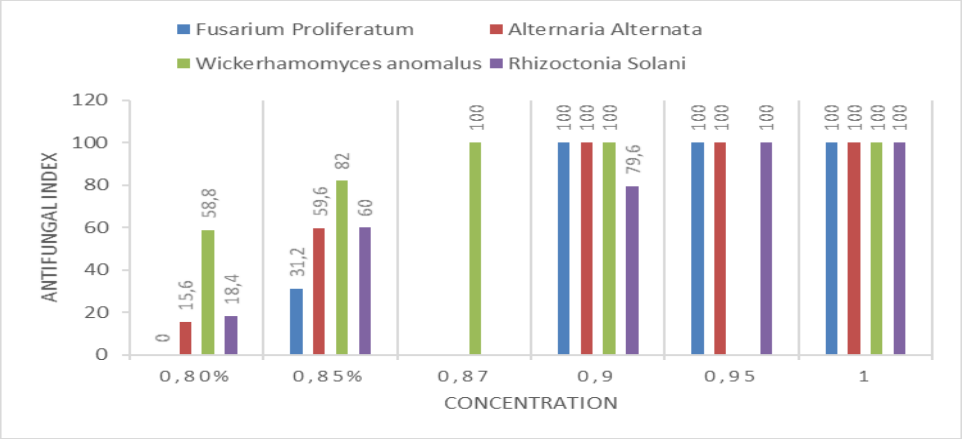

Figure 6: Antifungal index of inhibition of mycelial growth for different concentrations of *Mentha piperita* essential oil.

Mentha pulegium essential oil strongly inhibits the growth of strains **(Figure 7).** A 100% fungicidal inhibitory effect was observed at concentrations of 1% and 0.97% for all strains, and at 0.95% for *Wickerhamomycesanomalus* and *Rhizoctonia solani*. Effectiveness was recorded for *Fusarium proliferatum* and *Alternaria alternata* strains with an antifungal index of 83.6%. However, at concentrations of 0.80%, 0.85%, and 0.90%, the antifungal index increases as the concentration of the essential oil increases.

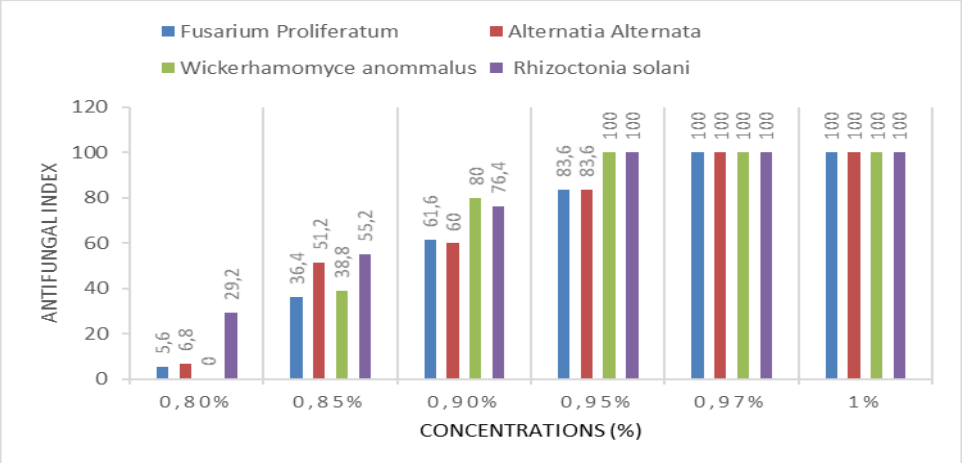

Figure 7: Antifungal index of inhibition of mycelial growth for different concentrations of *Mentha pulegium* essential oil.

On **Figure 8,** we observe a complete inhibition (100%) of mycelial growth for the fungal strains at concentrations of 0.97% and 1%. However, we obtained 16% inhibition at the

concentration of 0.80% for Alternaria alternata and no inhibition (0%) for *Fusarium proliferatum*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani*. At the concentration of 0.90%, we recorded 81.2% inhibition for *Fusarium proliferatum*, 72% for *Alternaria alternata,* 60.4% for *Wickerhamomyces anomalus*, and 66.8% for *Rhizoctonia solani*.

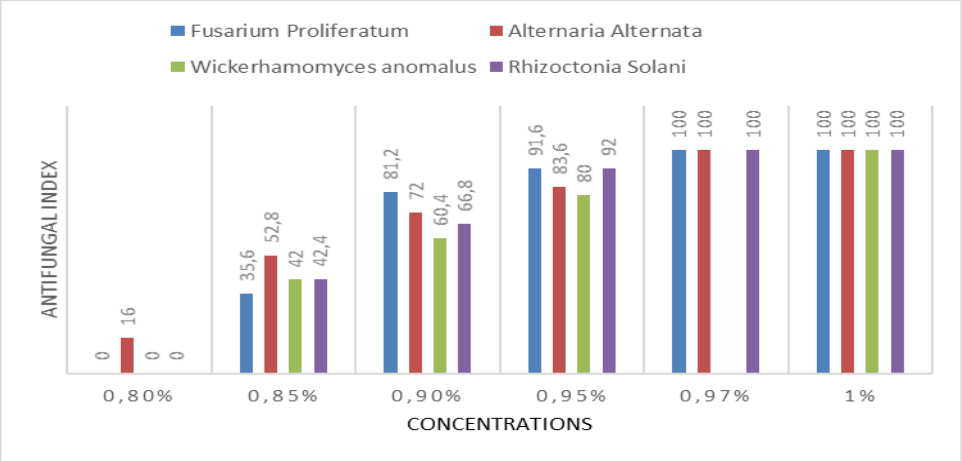

Figure 8: Antifungal index of inhibition of mycelial growth for different concentrations of *Ocimum basilicum* essential oil.

The effect of essential oils from *Mentha spicata, Mentha piperita, Mentha pulegium*, and *Ocimum basilicum* on the development of these phytopathogenic fungi varies according to the concentration. The lowest concentration completely inhibited the hyphal growth of the tested strains. This activity is likely due to the nature and molecular structure of the active ingredients in the essential oil. These compounds penetrate the cell membrane, enter the cell, interact with key intracellular sites such as enzymes and proteins, and induce cell death **(Omideygi *et al.*, 2007).**

The antifungal activity of the tested plant essential oils was found to be effective at all concentrations against the fungal strains. Thus, the inhibition rate increases with the decrease in hyphal growth until complete inhibition of the disc. Our results demonstrate some variability among the fungal strains in response to the essential oils. Specifically, *Alternaria alternata* and *Wickerhamomyces anomalus* are more sensitive than *Fusarium proliferatum* and *Rhizoctonia solan*i. The latter two strains likely exhibit inherent cellular resistance, and the difficulty in developing antifungal molecules has been identified as being related to the fungal cell's ultrastructure, which poses three barriers - the chitin cell walls, membrane ergosterol, and the eukaryotic nucleus **(Chami, 2005),** as well as the antifungal molecules themselves, which can generate resistance **(Prasad and Kapoor, 2005).**

The essential oil of *Mentha spicata* (spearmint) exhibits potent antifungal activity against the tested strains. This is supported by a study conducted by **Ismaili *et al.* (2014),** which demonstrated significant inhibition of dermatophyte growth. Similarly, in our study, the

essential oil of *Mentha piperita* (peppermint) shows strong antifungal activity against the tested strains. This is consistent with the findings of **Goujil *et al.* (2016),** who revealed that carvone, the main ingredient in peppermint essential oil, is considered responsible for its antifungal property. Furthermore, **Ferdes *et al.* (2012)** mentioned that essential oils from aromatic plants such as lemon and mint possess antifungal activity against other fungi such as *Aspergillus niger*, *Fusarium oxysporum*, *Monascus* purpureus, and *Penicillium hirsutum*. Additionally, peppermint oil was found to be the most effective against all the studied fungi.

The essential oil of *M. pulegium* (pennyroyal) also had an effect on the tested strains. **Lahlou *et al.* (2005)** demonstrated that the essential oil of this plant inhibited the growth of *Penicillium spp*. Similarly, **Hajlaoui *et al.*(2009)** reported that the pennyroyal oil from Tunisia, which is rich in menthone (61.11%), caused inhibition of cultures of Botrytis cinerea, *Fusarium culmorum*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, and *Trichoderma sp*. **Smid *et al.* (1995)** and **Hmiri *et al.* (2013)** studied the inhibitory effect of fifteen constituents of *M. pulegium* essential oil on the germination of *Penicillium hirsutum* conidia. Their results showed that pulegone, menthone, menthol, and carvone completely inhibited conidial germination, with carvone exhibiting the strongest inhibitory effect.

The essential oil of basil (*Ocimum basilicum*) induced complete inhibition of mycelial growth in the tested fungal strains. **Edris and Farrag (2003)** and **Doumouya et al. (2021)** found that the vapor of basil essential oil and its main compound, linalool, inhibited the growth of Mucor sp. and *Rhizopus stolonifer* in a dose-dependent manner. Additionally, **Awuah and Ellis (2002)** and **Doumouya *et al.* (2021)** demonstrated the effectiveness of basil leaf powder in protecting peanut stocks against *Aspergillus parasiticus* and stock contaminants.

The different observed inhibition rates suggest that the various essential oils have interesting antifungal effects against fungal strains associated with potato tuber rot. In general, the fungal strains showed sensitivity to increasing dosages, resulting in a progressive increase in the percentage of inhibition. Therefore, this inhibitory activity is "dose-dependent." The effectiveness of these oils could be attributed to their antifungal properties, which allow them to halt or slow down fungal mycelial production. According to **Kalemba and Kunicka (2003)** and **Doumouya *et al.* (2021),** the components of essential oils, such as terpenes and phenylpropanols, confer antibacterial and antifungal properties. Furthermore, the antifungal activity of essential oils can be attributed to the synergistic effect among their different compounds, with the major compounds often being responsible for the antifungal activity. However, minor components may also contribute significantly to the activity of essential oils.

Conclusion

The study focused on the antifungal activity of essential oils from four aromatic and medicinal plants cultivated in Algeria against four phytopathogenic fungi responsible for post-harvest

deterioration of potatoes. The analysis of these essential oils obtained by hydrodistillation revealed that peppermint (*Mentha piperita*), pennyroyal (*Mentha pulegium*), and spearmint (*Mentha spicata*) mainly contain carvone, while basil (*Ocimum basilicum*) is primarily composed of linalool. The tested essential oils clearly exhibited anti-growth effects on the studied fungi. They can be used as alternatives to synthetic fungicides in strategies for controlling pathogenic agents responsible for potato tuber rot while protecting the environment. These future bio-fungicides offer a promising approach in the fight against various fungal diseases.

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CHAPITRE III

Etude de l'activité antifongique des extraits aqueux de quatre plantes médicinales sur des champignons phytopathogènes isolés à partir de la pomme de terre (*Solanum tuberosum*) de la région d'El Oued (Sahara septentrional Est-algérien).

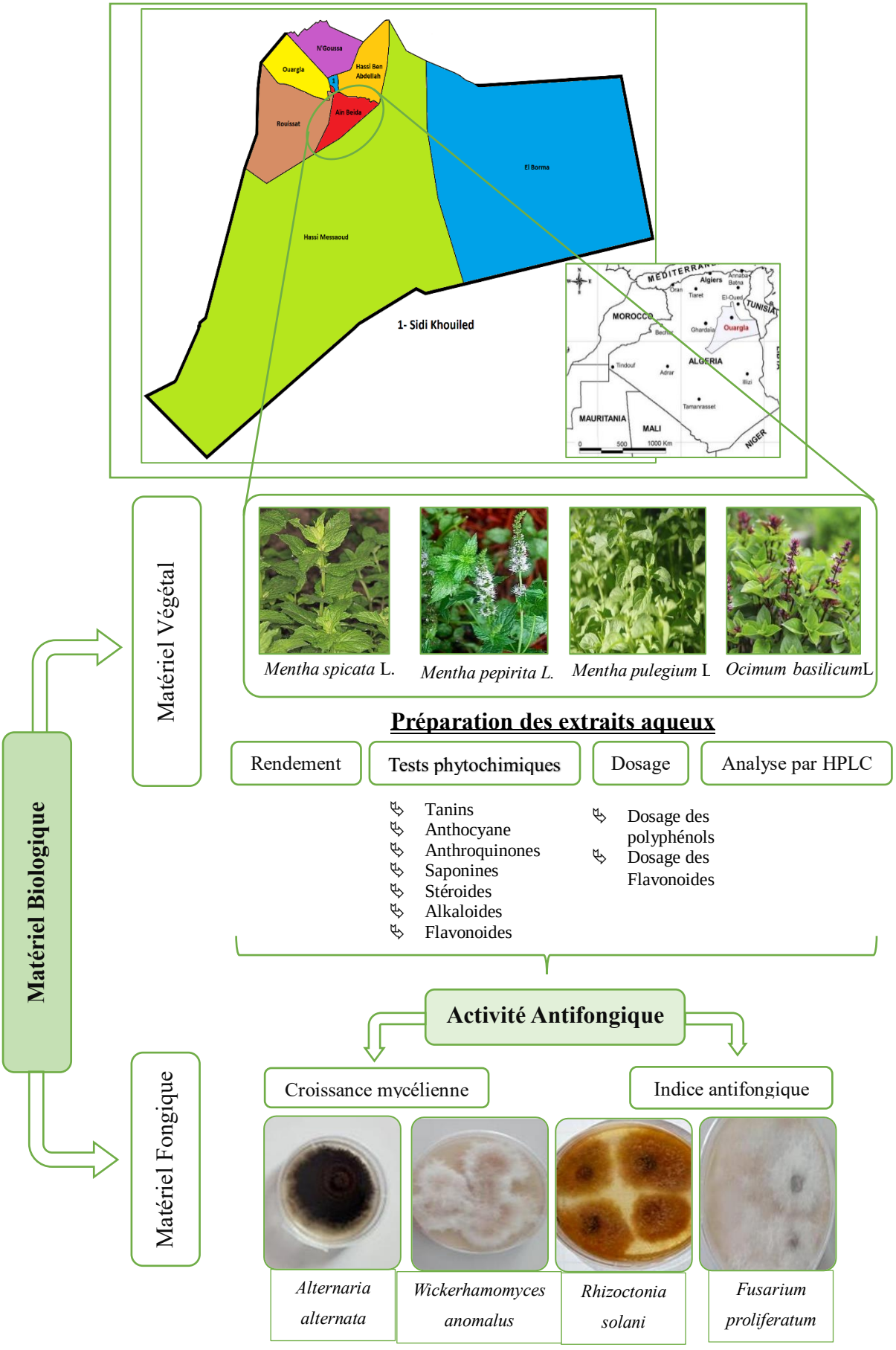

Figure 3 : Dispositif expérimental du Chapitre 3.

Study of the Antifungal Activity of Aqueous Extracts from Four Medicinal Plants on Phytopathogenic Fungi Isolated from Potato (*Solanum teberosum*) in the El Oued Region (Eastern Northern Sahara - Algeria) (Article publié en Juin 2024 dans la Revue du African Journal of Biological Sciences).

Chapitre III : Etude de l'activité antifongique des extraits aqueux de quatre plantes médicinales sur des champignons phytopathogènes isolés à partir de la pomme de terre (*Solanum tuberosum*) de la région d'El Oued (Sahara septentrional Est- algérien).

La méthodologie adoptée pour la réalisation de cette partie de travail est résumée dans la **Figure 3.**

Le présent chapitre a pour objectif d'analyser la composition chimique d'extraits aqueux provenant des parties aériennes de quatre plantes médicinales et cultivées : *Mentha spicata* L, *Mentha pepirita* L, *Mentha pulegium* L et *Ocimum basilicum* L **(voir annexe I)**. et d'évaluer leur action antifongique sur certains champignons phytopathogènes, *Alternaria alternata, Wickerhamomyces anomalus, Fusarium proliferatum* et *Rhizoctonia solani,* isolés à partir de tubercules de pomme de terre en post-récolte de la région d'El Oued (Sahara septentrional Est algérien) dont le numéro d'accès GenBank NCBI (Centre national d'information sur la biotechnologie) est OQ606246.1, OQ860003.1, OQ771178.1 et OQ606247.1

La macération sous agitation pendant 24 heures a été utilisée pour obtenir les extraits aqueux. Les résultats ont révélé que le rendement des plantes varie entre 0,13 -0,27% **(tableau 2, article).** Les tests phytochimiques effectués sur les extraits aqueux permettent d'obtenir une vision qualitative de la présence ou de l'absence de molécules bioactives, retrouvées au niveau des quatre extraits aqueux. Les extraits des plantes étudiées sont riches en métabolites secondaires dont les alcaloïdes, les tanins, les saponosides et les flavonoides, **(tableau 3, article)**. La teneur en composés phénoliques totaux des différents extraits de plantes était comprise entre 3,277 et 11,124 mg, tandis que la teneur en flavonoïdes totaux des différents extraits de plantes était comprise entre 0,896 et 2,312 mg **(tableau 4, article)**.

L'analyse par HPLC (au niveau de laboratoire CRAPC, Ouargla) **(voir annexe II)** a révélé que l'extrait aqueux de *M. piperita* est composé d'acide acétylsalicylique, d'acide chlorogénique et d'acide *para* comarique, Par contre *M. pulegium* est caractérisée par la présence d'acide acétylsalicylique, de caféine et de pyrogallol. L'*O. basilicum* contient de l'acide gallique.

Les extraits aqueux ont été testés pour leur activité antifongique en utilisant la méthode du contact direct contre la croissance mycélienne des souches testées. Les résultats ont révélé

un fort potentiel d'activité antifongique envers ces souches. Aux concentrations 1,5%, 2%, 3,5% et 4%, l'extrait aqueux de *Mentha spicata* a présenté un effet antifongique de 100% sur les 4 champignons (**Figure 5, article**). Concernant *Mentha pepirita*, (**Figure 6, article**) l'extrait aqueux à 1%, a provoqué une inhibition totale de 100% chez *Alternaria alternata* et *Wickerhamomyces anomalus*, et à 4% chez *Fusarium proliferatum* et *Rhizoctonia solani*. Nous avons observé un effet inhibiteur fongicide (100 %) à 1,5 % chez *Alternaria alternata* et *Wickerhamomyces anomalus*, et à 3,5% chez *Fusarium proliferatum* et *Rhizoctonia solani* pour l'extrait aqueux de *Mentha pulegium* (**Figure 7, article**).

L'extrait aqueux d*'Ocimum bacilicum* (**Figure 8, article**) a entrainé une inhibition totale (100%) aux concentrations 2%, 2.5%, 5.5% et 7.5% chez *Wickerhamomyces anomalus*, *Alternaria alternata*, *Fusarium proliferatum* et *Rhizoctonia solani* respectivement.

Les résultats obtenus ouvrent la voie à l'exploitation des parties aériennes de plantes investiguées comme alternative aux fongicides chimiques de synthèse.

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Study of the Antifungal Activity of Aqueous Extracts from Four Medicinal Plants on Phytopathogenic Fungi Isolated from Potato (*Solanum teberosum*) in the El Oued Region (Eastern Northern Sahara - Algeria)

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Abstract

In this study, our objective was to analyze the chemical composition of aqueous extracts from the aerial parts of four plants: *Mentha spicata* L., *Mentha piperita* L., *Mentha pulegium* L., and *Ocimum basilicum* L., and to evaluate their antifungal activity against certain phytopathogenic fungi, specifically *Alternaria alternata*, *Wickerhamomyces anomalus*, *Fusarium proliferatum*, and *Rhizoctonia solani*, which were isolated from post-harvest potato tubers from the El Oued region (northern Sahara, eastern Algeria).

Maceration with agitation for 24 hours was employed to obtain the aqueous extracts. The results revealed that the yield varied between 0.13% and 0.27%. Phytochemical tests conducted on the aqueous extracts highlighted the presence of various chemical compounds such as tannins, flavonoids, saponins, and alkaloids. The content of total phenolics of different plants extracts was in the range of 3.277 - 11.124 mg. while the content of total flavonoids of the different plants extracts was in the range of 0.896 - 2.312 mg. HPLC analysis revealed that the aqueous extract of *M. piperita* is composed of acetylsalicylic acid, chlorogenic acid, and p-coumaric acid, whereas *M. pulegium* is characterized by the presence of acetylsalicylic acid, caffeine, and pyrogallol. *O. basilicum* contains gallic acid.

The aqueous extracts were tested for their antifungal activity using the direct contact method against the mycelial growth of the tested strains. The results revealed a strong antifungal potential against these strains. At concentrations of 1.5%, 2%, 3.5%, and 4%, the aqueous extract of *Mentha spicata* exhibited a 100% antifungal effect on all four fungi. Regarding *Mentha piperita*, the aqueous extract at 1% caused total inhibition (100%) of *Alternaria alternata* and *Wickerhamomyces anomalus*, and at 4% for *Fusarium proliferatum* and *Rhizoctonia solani*. We observed a fungicidal inhibitory effect (100%) at 1.5% for *Alternaria alternata* and *Wickerhamomyces anomalus*, and at 3.5% for *Fusarium proliferatum* and *Rhizoctonia solani* with the aqueous extract of *Mentha pulegium*. The aqueous extract of *Ocimum basilicum* resulted in total inhibition (100%) at concentrations of 2%, 2.5%, 5.5%, and 7.5% for *Wickerhamomyces anomalus*, *Alternaria alternata*, *Fusarium proliferatum*, and *Rhizoctonia solani* respectively.

The results obtained pave the way for the exploitation of the aerial parts of the investigated plants as an alternative to synthetic chemical fungicides.

Keywords: Potato, medicinal plants, phytopathogenic fungi, aqueous extracts, antifungal activity.

1. Introduction

The potato (*Solanum tuberosum* L.), belonging to the Solanaceae family, is the fourth most important staple food in the world, following wheat, rice, and maize. According to the United Nations Food and Agriculture Organization (FAO), this crop covers more than 17 million hectares worldwide, with global production exceeding 370 million tons **(FAOSTAT, 2021).**

The significance of the potato among staple crops lies in its highly nutritious properties, its potential for both industrial and domestic use, and its accessibility to consumers from low-income backgrounds **(Zaheer and Akhtar, 2016).**

Each year, a considerable portion of global agricultural production is lost due to various pests and diseases, which have a significant impact on agricultural yields **(Whish et al., 2014).** The majority of these diseases are caused by harmful fungi, accounting for approximately 20 to 40% of losses **(McDonald and Stukenbrock, 2016)**.

To minimize post-harvest losses, perishable products are treated with synthetic chemical fungicides. However, some of these fungicides are carcinogenic, highly toxic, have long degradation periods, and cause environmental pollution. They pose a threat to human safety, thereby necessitating a growing shift towards agriculture free from chemical residues (**Kamele et al., 2019).**

Furthermore, chemical fungicides can lead to severe medical complications, including various types of cancer. Thus, it is essential to explore natural antifungal substances as alternative methods to combat plant diseases **(Da et al., 2019).**

Among natural resources, plant extracts from medicinal and aromatic plants may contain a variety of biologically active molecules that act directly on microbial pathology. These extracts offer advantages (medical or phytosanitary) that the commonly used fungicides often lack **(Senhaji et al., 2005; Mahesh and Satish, 2008).**

In this context, we aimed to evaluate the antifungal activity of certain extracts from medicinal and aromatic plants cultivated in southern Algeria against phytopathogenic fungi affecting potatoes.

2. Materials and Methods

2.1. Plant Material

The plants used in this study belong to the Lamiaceae family, consisting of spearmint (*Mentha spicata* L.), peppermint (*Mentha piperita* L.), pennyroyal (*Mentha pulegium* L.), and basil (*Ocimum basilicum* L.). In August 2020, the peak production period for these plants, the aerial parts were harvested in the region of Ouargla (southeastern Algeria), at the coordinates (N31°57'47" E5°20'31").

2.2.Fungal Material

In this study, four phytopathogenic fungal strains were isolated from potato tubers originating from the El Oued region (N33°07', E7°11'). The fungal species used were *Fusarium*

proliferatum, *Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani*. These strains were purified and identified by PCR, with GenBank accession numbers NCBI (National Center for Biotechnology Information) OQ606246.1, OQ860003.1, OQ771178.1, and OQ606247.1 **(Benhaoued et al., 2024).**

2.3. Preparation of Aqueous Plant Extracts

The aqueous extracts were prepared by macerating 10 g of plant powder in 100 ml of distilled water, with agitation at 200 rpm for 24 hours at a temperature of 25°C **(Razak et al., 2009; Beddou et al., 2015).** The mixture was then filtered using Whatman filter paper and centrifuged at 600 rpm for 15 minutes. The recovered filtrate was evaporated to dryness under reduced pressure at 40°C using a rotary evaporator. The yield (in %) was determined using the following formula **(Majhenic, 2007)**

$$\mathbf{Rd\ \% = (m_1\ X\ 100)\ /\ m_0}$$

Where:

- Rd: Yield

- (m_1): Mass of the dry extract (in g)

- (m_0): Mass of the dry plant material (in g)

2.4. Phytochemical Tests

A phytochemical study involves characterizing the different categories of molecules present in a plant **(Bruneton, 1999)**. To achieve this, the various obtained extracts were subjected to phytochemical tests. These tests are based on color reactions and precipitation **(Bruneton, 1999; Mojab et al., 2003; Karumi et al., 2004; Oloyede, 2005; Koffi et al., 2009).**

2.5. Quantitative Analysis

2.5.1. Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent was used to measure the total phenolic content (TPC) of the different extracts (**Singleton and Rossi, 1965**). Gallic acid (0.03-0.25 g/L) was used as a standard reference to construct the calibration curve. 0.1 ml of each extract was mixed with 0.5 ml of 10% Folin-Ciocalteu reagent. After 2 minutes, mixture was neutralized with 2 ml of 20% Na_2CO_3 solution. The reaction mixture was kept in the dark at 25°C for 30 minutes. The absorbance of the blue color was measured using a UV/Vis spectrophotometer at a fixed wavelength of 760 nm. TPC was calculated using the linear regression equation obtained from the gallic acid standard curve. The total phenolic content was calculated as the mean ± SD (n = 3) and expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (DW).

2.5.2. Determination of Total Flavonoid Content (TFC)

The total flavonoid content was estimated according to a procedure described in **(Belguidoum et al., 2015)** protocol. 1.5 ml of an ethanolic solution of $AlCl_3$ (2%) was added to 1.5ml of each extract. After 30 minutes at room temperature, the absorbance was measured

at 430 nm. The total flavonoid content was calculated as the mean ± SD (n = 3) and expressed as mg of quercetin equivalents (QE) per gram of dry weight (DW).

2.6. Characterization of Polyphenols in Aqueous Extracts

The qualitative analysis of phenolic compounds in the aqueous extracts was performed using high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) under the conditions listed in (**Table 1)**.

Table 1: Conditions for High-Performance Liquid Chromatography (HPLC) Coupled with Mass Spectrometry (MS).

Time	65.00 min
Volume injection	10 ul
Max Press	40.0 MPa
Press Min	0.0 MPa
Maximum Temperature	90° C
Mobile phase	A (ultra-pure water) B (Ethanol)
Processed by	HPLC (SHUMADZU)
Column Name	Ultra C18
Column ID	250 x 4.6 mm
Particle Size	5 A

2.7. Antifungal Activity

2.7.1 Direct Contact Methods

We employed the direct contact method **(Mohammedi et al., 2012)** to evaluate the antifungal efficacy of the aqueous plant extracts.

2.7.1.1. Mycelial Growth

Mycelial growth was measured (in mm) at the end of the experiment, after 10 days of incubation (240 hours). Measurements were taken from the average of three perpendicular diameters. These readings were always compared to control cultures that started on the same day and under the same conditions.

2.7.1.2. Determination of the Antifungal Index

According to **Kordali et al. (2003),** the antifungal index for each aqueous extract is calculated by the reduction in the fungal colony diameter relative to the control parameter, using the following formula:

$$I\ (\%) = [1 - (D_{test} / D_{Control})] \times 100$$

Where:

$D_{control}$: Mycelial diameter growth in a medium without the presence of the aqueous extracts (in mm).

D_{test}: Mycelial diameter growth in the presence of the aqueous extracts (in mm).

2.8. Statistical Analysis

The means, standard deviations, and analysis of variance (ANOVA) were calculated from the obtained results for each parameter using XLSTAT software (2019).

3. Results and Discussion

3.1. Extraction and Yields

The yields of the aqueous extracts obtained after maceration and evaporation of the 4 tested plants are presented in (**Table 2).**

Table 2: Yields of Aqueous Extracts from the Studied Plants

Space	*Mentha spicata*	*Mentha piperita*	*Mentha pulegium*	*Ocimum basilicum*
Yields (%)	0.27% ±0.01	0.13% ±0.01	0.21% ±0.01	0.17% ±0

The data analysis from(**Table 2)** reveals that the yields of the aqueous extracts vary according to the plant species and range between 0.13% and 0.27%. The yield of *Mentha spicata* is 0.27%, which is lower than that reported by **Zekri et al. (2021)** who recorded 4.9%. **Abkhoo and Jahani (2017)** indicated that the yields of plant extracts vary depending on the extraction solvent and the plant extract used. Their results showed that the yields of aqueous extracts of *Mentha spicata* were 23.8%. **Kaddour et al. (2022)** mentioned respective yields of aqueous extract of *Mentha spicata* from the regions of El-Oued, Tibessa, and El-Tarf as 13.4%, 12.7%, and 11%. The yield of *Mentha piperita* (0.13%) is lower than that reported by **Dorman et al. (2009)** who obtained 1.29%. The yield of aqueous extracts of *Mentha pulegium* (0.21%) is lower than that mentioned by **Zekri et al. (2021)** who recorded 3.8% for this species. **Khennouf et al. (2013)** obtained a yield of 14.4% from the methanolic extract of *Mentha pulegium*. The yield of *Ocimum basilicum* is 0.17%.

3.2. Phytochemical Screening

The results of the phytochemical characterization tests of the aqueous extracts of *Mentha spicata*, *Mentha piperita*, *Mentha pulegium*, and *Ocimum basilicum* are presented in (**Table 3)**. These results provide a qualitative insight into the presence or absence of bioactive molecules found in the four aqueous extracts.

Table 3: Chemical screening of plants extracts

Compounds	*Mentha spicata*	*Mentha piperita*	*Mentha pulegium*	*Ocimum basilicum*
Tannins	+	+	+	+
Anthocyanins	-	-	-	-
Anthroquinones	+	+	-	-
Saponnosides	+	+	+	+
Steroides	-	-	-	-
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+

- : Absence + : Presence

The extracts of the studied plants are rich in secondary metabolites including alkaloids, tannins, saponins, and anthraquinones. The aqueous extract of *Mentha spicata* is rich in flavonoids, saponins, alkaloids, tannins, and anthraquinones. **EL-Haoud et al. (2018)** indicated that the aqueous extract of *Mentha spicata* is very rich in polyphenols with a significant presence of cardiac glycosides. Sterols, terpenes, and flavonoids are moderately present in the aqueous extract, while reducing compounds and alkaloids were not determined. **Ullah et al. (2011)** reported that spearmint from four regions of Pakistan contains tannins, alkaloids, flavonoids, coumarins, sterols, and triterpenes. However, saponins and anthraquinones were not detected. Similarly, the study by **Naidu et al. (2012)** revealed the presence of alkaloids, flavonoids, and glucosides in the crude extract of this species. Conversely, the study by **Prasad et al. (2010)** mentioned the absence of phenols, saponins, flavonoids, cardiac glycosides, and terpenes.

The aqueous extract of *Mentha piperita* is rich in secondary metabolites such as alkaloids, saponins, flavonoids, and tannins. **Khamis and Aly (2017)** detected the presence of glycosides, flavonoids, and tannins in peppermint extract, while alkaloids, saponins, phenols, steroids, and proteins were not detected **Patil et al. (2016)** indicated that the aqueous extract of *Mentha piperita* contains diterpenes, steroids, tannins, flavonoids, carbohydrates, alkaloids, phenols, coumarin, and saponins, except for anthocyanins. **Muhammad et al. (2019)** mentioned the

presence of flavonoids, terpenoids, saponins, and phenols in the aqueous extract, except for glycosides, steroids, alkaloids, and tannins.

The phytochemical screening of the aqueous extract of *Mentha pulegium* also shows richness in secondary metabolites (alkaloids, flavonoids, saponins, and tannins). According to the study by **Rao and Tiwari (2021)**, the phytochemical screening of the extract of *M. pulegium* from the stem, leaves, and inflorescences revealed the presence of alkaloids, flavonoids, steroids, phenols, terpenoids, and tannins.

As for the extract of *Ocimum basilicum*, we detected the presence of tannins, saponins, and alkaloids. **Khamis and Aly (2017)** mentioned the presence of glycosides, phenols, flavonoids, alkaloids, saponins, steroids, proteins, tannins, and terpenoid carbohydrates. According to **Jacob et al. (2016)**, the phytochemical test of aqueous extracts of *O. basilicum* showed the presence of alkaloids, saponins, flavonoids, tannins, glycosides, steroids, and terpenoids.

3.3. Total phenolic content (TPC) and Total Flavonoid Content (TFC)

Table 4 : Total phenolic content (TPC) and Total Flavonoid Content (TFC) of plant extracts

Sample	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Mentha spicata	3.277±0.492	1.435±0.011
Mentha piperita	7,646±1,401	2.312±0.318
Mentha pulegium	5,447±0.965	0.896±0.075
Ocimum Bacilicum	11,124±1,265	1.824±0.133

The TPC of extracts varied from 3.277 to 11.124 mg of gallic acid equivalents (GAE)/g of sample DW **(Table 4)**. The highest TPC was obtained in the *Ocimum basilicum* extract, while the lowest TPC was found in the *Mentha spicata* extract. The order of TPC is: *Mentha spicata* < *Mentha pulegium* < *Mentha piperita* < *Ocimum basilicum* (**Table 4).**This study showed that the extracts have a low phenolic content compared to other studies, such as those of **Bahman et al., 2008** 150.91–433.60 mg GAE/g DW, **Politeo et al 2018**. 124.27 mg GAE/g DW, and **Karra-Bouraoui et al., 2009**. 20.1–64.5 mg GAE/g DW. D. **Gajula et al., 2009**. reported 31.37–60.47 mg GAE/g DW for *Ocimum basilicum*.

The TFC of extracts varied from 0.896 to 2.312 mg of quercetin equivalents (QE)/g of sample DW **(Table 04).** The highest TFC was obtained in the *Mentha piperita* extract, while the lowest TFC was found in the *Mentha pulegium* extract. The order of TFC is: *Mentha pulegium* < *Mentha spicata* < *Ocimum basilicum* < *Mentha piperita* (**Table 4**). This study showed that the extracts have a low flavonoid content compared to other studies, such as those of **Olivera Politeo et al**., **2018**. 12.70 mg QE/100 g DW and **Karra-Bouraoui et al., 2009**. 12.9–53.3 mg catechin E/g DW. D. **Gajula et al., 2009**. reported 4.64–6.21 mg catechin E/g DW for *Ocimum basilicum.*

3.4. HPLC Chromatography

The aqueous extract of *Mentha spicata* yielded no results when compared with the standard. According to the work of **Cirlini et al. (2016)** on the phenolic composition of *Mentha spicata* extract, HPLC analysis revealed that the extract is composed of rosmarinic acid and its derivatives with smaller quantities of salvianolic acids, caffeoylquinic acids, hydroxybenzoic acids, hydroxycinnamic acids, flavones, and flavanones.

The aqueous extract of *Mentha piperita* contains the following compounds: acetylsalicylic acid, chlorogenic acid, *para*-coumaric acid, and vitamin B1. **Dorman et al. (2009)** mention that the polyphenolic composition of aqueous extract of *Mentha piperita* contains three main polyphenols, among which are eriocitrin, luteolin-7-O-glucoside, and rosmarinic acid.

The aqueous extract of *Mentha pulegium* is composed of acetylsalicylic acid, caffeine, and pyrogallol. **Alharbi et al. (2021)** indicate the presence of seven polyphenolic compounds in the methanolic extract of *M.pulegium* such as eriocitrin, hesperidin, narirutin, luteolin, isorhoifolin, rosmarinic acid, and caffeic acid.

The aqueous extract of *Ocimum basilicum* is composed of gallic acid, while **Kanmaz et al. (2023)** detected the presence of 24 phenolic compounds. Among the main compounds found were t-caffeic acid, caftaric acid, kaempferol-3-glucoside, and quercetin-3-glucoside.

Our study conducted on the aqueous extracts of *Mentha spicata*, *Mentha piperita*, *Mentha pulegium*, and *Ocimum basilicum* revealed partially similar results to other works, namely the presence of certain chemical families, but also the absence of other chemical compounds. This can be explained by differences in several parameters, which can be geographical, physicochemical, or biological, such as the collection site, including the plant's environment, light, precipitation, topography, season, soil type, harvesting period, genetic heritage, extraction procedure used, and the plant part studied **(Malik et al., 2012; Sujana et al., 2013; Akhtar et al., 2018).**

3.5. Mycelial Growth

The antifungal activity is revealed by the absence or presence of mycelial growth in fungal strains.

3.5.1.*Mentha spicata*

The analysis of variance indicates a highly significant difference ($p<0.0001$) for the mycelial growth of the tested fungal strains.

In **Figure 1**, we can observe the impact of the aqueous extract of *Mentha spicata* on the mycelial growth of tested fungi at different doses. A maximum development (50mm) of hyphae was observed in all controls. The same applies to *Fusarium proliferatum* and *Rhizoctonia solani* at concentrations of 0.90%, 0.95%, 1%, and 1.5% for *Rhizoctonia solani*. However, with increasing concentrations, mycelial growth decreases compared to the control, eventually leading to complete inhibition. *Alternaria alternata* and *Wickerhamomyces anomalus* show a decrease in growth starting from the concentration of 0.90%, with respective diameters of

35.5mm and 41mm, reaching complete inhibition of their growth at concentrations of 1.5% for *Alternaria alternata* and 02% for *Wickerhamomyces anomalus*. No mycelial growth was obtained at a concentration of 4% for all strains. The Minimum Inhibitory Concentrations (MIC) are 3.5% for *Fusarium proliferatum*, 1.5% for *Alternaria alternata*, 2% for *Wickerhamomyces anomalus*, and 4% for *Rhizoctonia solani*.

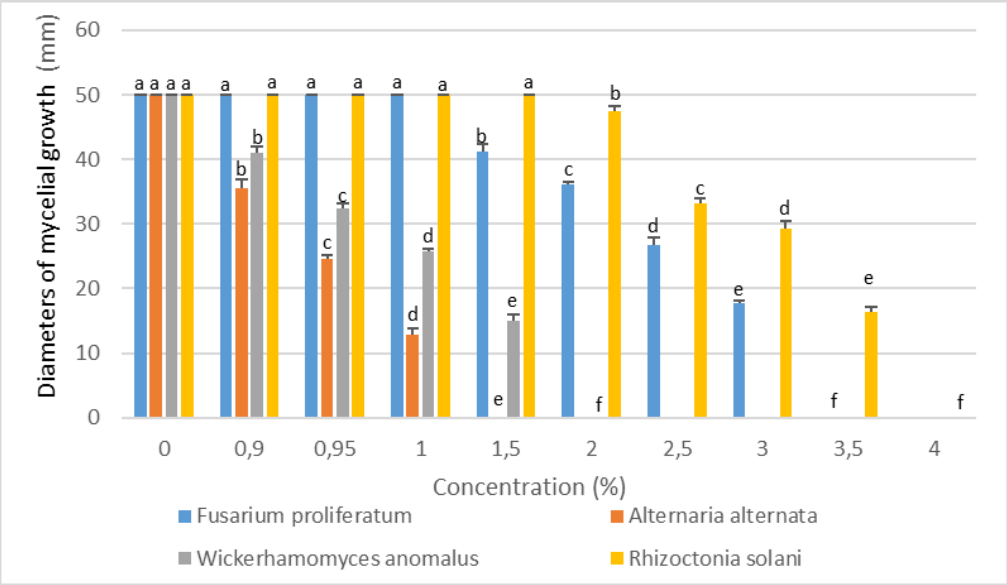

Figure 1: Effect of different concentrations of aqueous extracts of *Mentha spicata* on mycelial growth.

3.5.2.*Mentha piperita*

The analysis of variance indicates a highly significant difference ($p<0.0001$) for the mycelial growth of the tested fungal strains.

The results in **Figure 2** show a maximum diameter (50mm) of mycelial growth for the 4 fungal strains in the controls, corresponding to the absence of the aqueous extract as well as concentrations of 0.80% and 0.85%, except for *Wickerhamomyces anomalus*, which decreases in diameter to 47.5mm. The same pattern is observed at concentrations ranging from 0.90% to 1.5% for *Fusarium proliferatum* and *Rhizoctonia solani*. The mycelial growth of the fungi slightly decreases as the concentration of the aqueous extract increases, eventually leading to growth inhibition. The Minimum Inhibitory Concentrations (MIC) for *Fusarium proliferatum* and *Rhizoctonia solani* are 4%, while *Alternaria alternata* and *Wickerhamomyces anomalus* have a (MIC) of 1%.

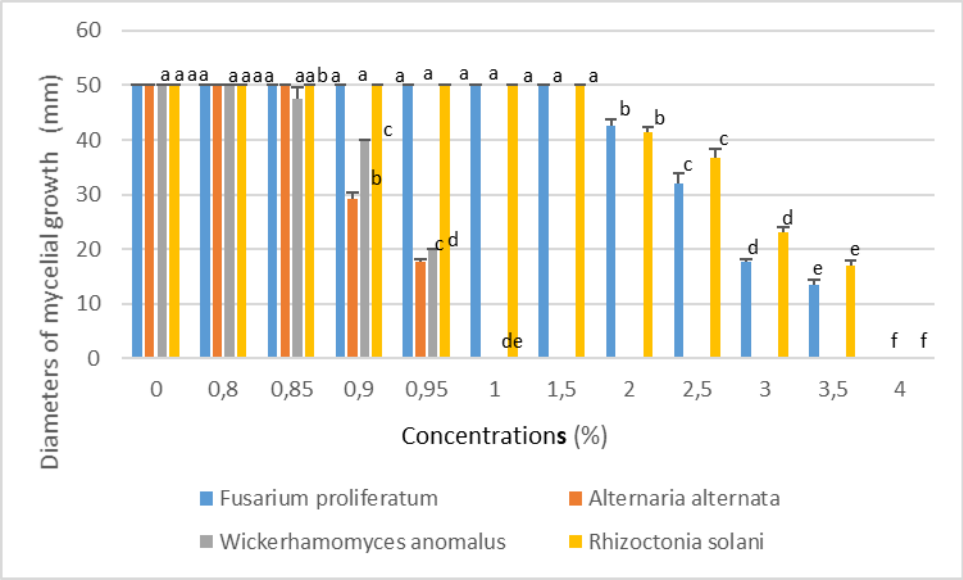

Figure 2: Effect of different concentrations of aqueous extracts of *Mentha piperita* on mycelial growth.

3.5.3.*Mentha pulegium*

Figure 3 presents the results of the effects of the aqueous extract of *Mentha pulegium* on the growth of tested fungal species. The analysis of variance indicates a highly significant difference ($p<0.0001$) for the mycelial growth of the tested fungal strains.

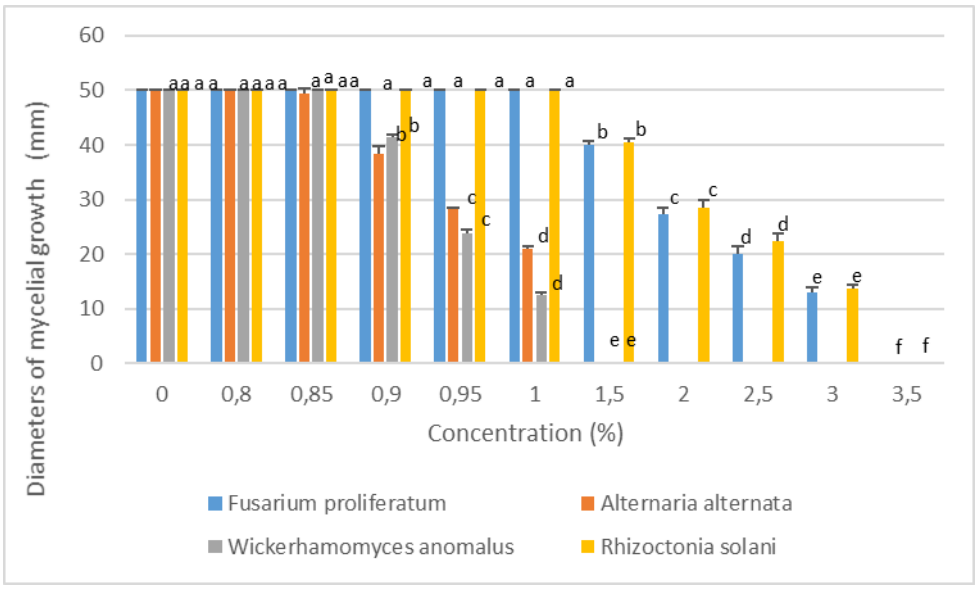

Figure 3: Effect of different concentrations of aqueous extracts of *Mentha pulegium* on mycelial growth.

According to **Figure 3**, we recorded a maximum diameter (50mm) of mycelial growth in the 4 untreated fungal strains (control) as well as at concentrations of 0.80% and 0.85%. The same

result was also obtained for *Fusarium proliferatum* and *Rhizoctonia solani* at concentrations of 0.90%, 0.95%, and 1%. The mycelial growth slightly decreases with increasing concentration of the aqueous extract of *Mentha pulegium*, eventually leading to complete inhibition of growth at 1.5% for *Alternaria alternata* and *Wickerhamomyces anomalus*, and at 3.5% for *Fusarium proliferatum* and *Rhizoctonia solani*. The Minimum Inhibitory Concentrations (MIC) .

3.5.4.*Ocimum basilicum*

The analysis of variance indicates a highly significant difference ($p<0.0001$) for the mycelial growth of the tested fungal strains.

Based on the results depicted in **Figure 4**, we observed a maximum mycelial growth diameter of 50mm in all fungal strains at concentrations of 0% (control) and 0.85%. This result is also observed for *Fusarium proliferatum* and *Rhizoctonia solani* in the range of [0-2.5%], as well as for *Rhizoctonia solani* at concentrations of 3% and 3.5%. As the concentration increases, the mycelial diameter of the fungal strains decreases, eventually leading to complete growth inhibition (0mm) at concentrations of 2% for *Wickerhamomyces anomalus*, 2.5% for *Alternaria alternata*, and 5.5% for *Fusarium proliferatum*. *Rhizoctonia solani* shows resistance up to a concentration of 7.5% (Minimum Inhibitory Concentration).

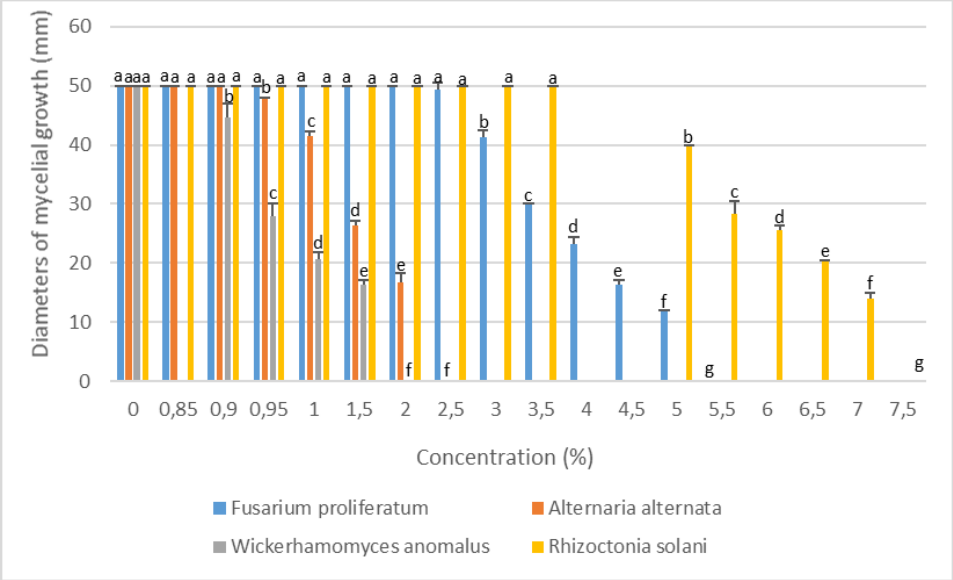

Figure 4: Effect of different concentrations of aqueous extracts of *Ocimum basilicum* on mycelial growth.

3.6. Antifungal Index

3.6.1. *Mentha spicata*

The action of the aqueous extract of *Mentha spicata* on the growth of different fungal strains is variable **Figure 5.** We observe an increase in the antifungal index as the concentration of the extract increases. Thus, we obtained average inhibition rates of 50.8%, 48.4%, 46.4%, and 41.6% at respective concentrations of 0.95%, 1%, 2.5%, and 3% for *Alternaria alternata*, *Wickerhamomyces anomalus*, *Fusarium proliferatum*, and *Rhizoctonia solani*, respectively. A

100% inhibition rate was observed at concentrations of 1.5%, 2%, 3.5%, and 4% for *Alternaria alternata*, *Wickerhamomyces anomalus*, *Fusarium proliferatum*, and *Rhizoctonia solani*, respectively.

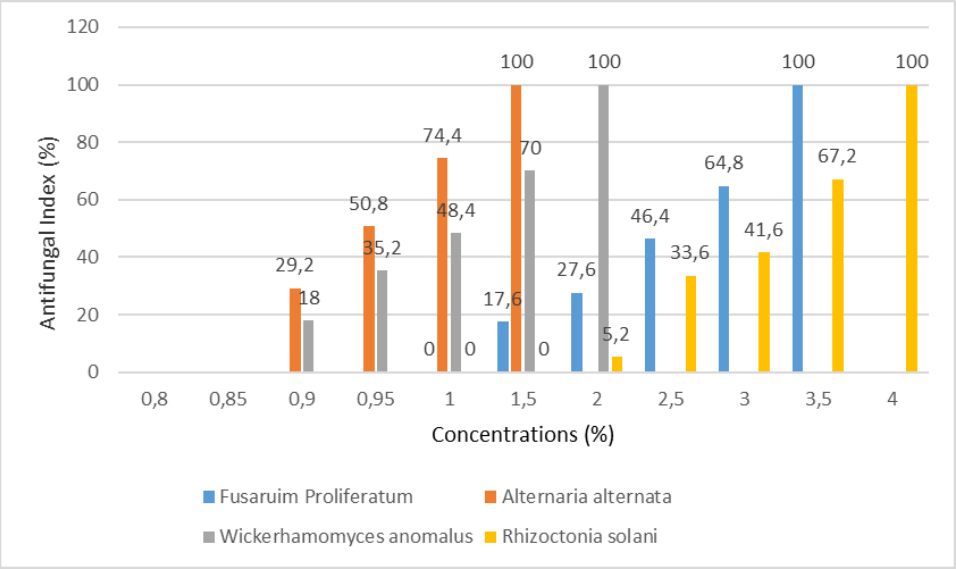

Figure 5: Antifungal Index showing inhibition of mycelial growth according to the concentration of aqueous extract of *Mentha spicata*.

3.6.2. *Mentha piperita*

Figure 6 represents the inhibition rate of mycelial growth for the tested fungal strains at different concentrations of aqueous extract of *Mentha piperita*. We observe that the antifungal index increases with the elevation of the extract concentration. An average inhibition rate of 64.8% and 60% is recorded at a concentration of 0.95% for *Alternaria alternata* and *Wickerhamomyces anomalus*, respectively. Regarding *Fusarium proliferatum* and *Rhizoctonia solani*, they exhibit respective average inhibition rates of 64.8% and 54% at a concentration of 3%. A total inhibition rate of 100% is recorded at concentrations of 1% for *Alternaria alternata* and *Wickerhamomyces anomalus*, and at 4% for *Fusarium proliferatum* and *Rhizoctonia solani*.

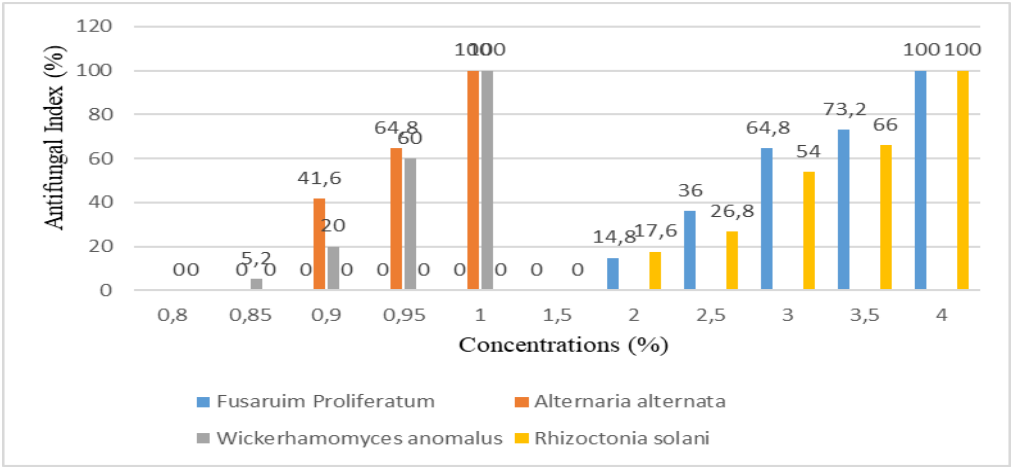

Figure 6: Antifungal Index showing inhibition of mycelial growth according to the concentration of aqueous extract of *Mentha piperita*.

3.6.3. *Mentha pulegium*

According to **Figure 7**, we observe an increase in the antifungal index as the concentration increases. We recorded respective average inhibition rates of 42.8% and 52.4% at a concentration of 0.95% for *Alternaria alternata* and *Wickerhamomyces anomalus*, and 59.8% and 55.2% at a concentration of 2.5% for *Fusarium proliferatum* and *Rhizoctonia solani*, respectively. A 100% inhibitory and fungicidal effect was observed at a concentration of 1.5% for *Alternaria alternata* and *Wickerhamomyces anomalus*. As for *Fusarium proliferatum* and *Rhizoctonia solani*, complete inhibition was only achieved at a concentration of 3.5% of the aqueous extract.

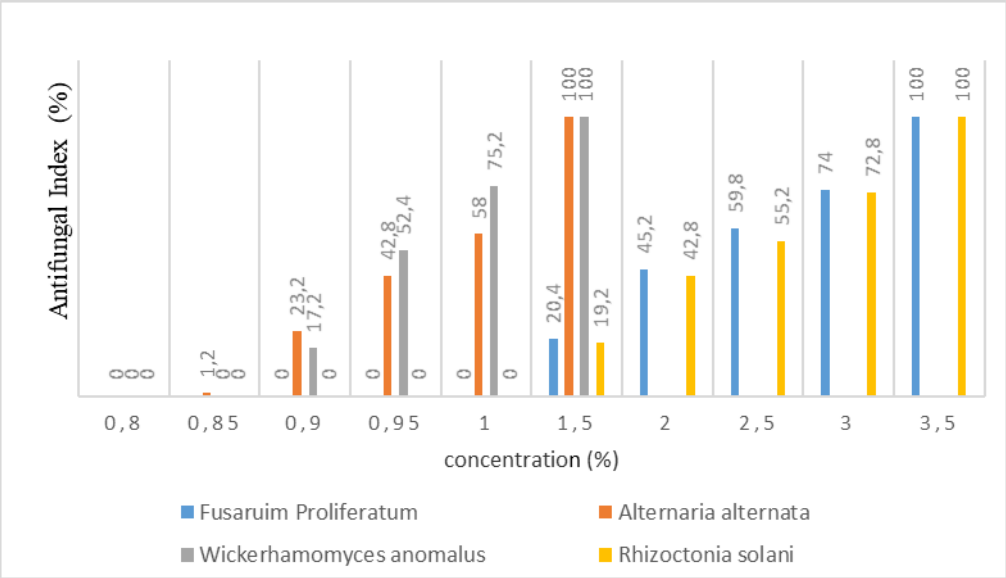

Figure 7: Antifungal Index showing inhibition of mycelial growth according to the concentration of aqueous extract of *Mentha pulegium*.

3.6.4. *Ocimum basilicum*

According to the results mentioned in **Figure 8**, we observe that the mycelial growth of fungi is inhibited with the increase in concentrations of the aqueous extract of *Ocimum basilicum.* Thus, we recorded average inhibition rates of 53.6%, 44%, 47.2%, and 49.2% at concentrations of 4%, 0.95%, 1.5%, and 6% respectively for *Fusarium proliferatum*, *Wickerhamomyces anomalus*, *Alternaria alternata*, and *Rhizoctonia solani*. As for the antifungal indices, we recorded inhibition rates of 100% at respective concentrations of 2%, 2.5%, 5.5%, and 7.5% for *Wickerhamomyces anomalus*, *Alternaria alternata*, *Fusarium proliferatum*, and *Rhizoctonia solani*.

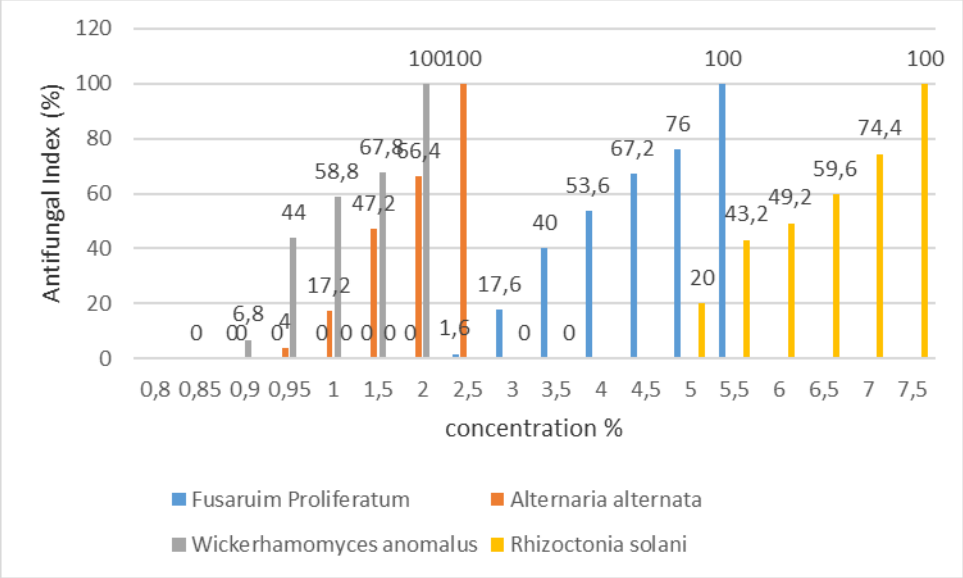

Figure 8: Antifungal Index showing inhibition of mycelial growth according to the concentration of aqueous extract of *Ocimum basilicum*.

The use of new natural substances as alternatives to chemical agents remains a promising solution for crop protection. The antifungal activities of the tested plant extracts against four fungal strains responsible for post-harvest diseases in potatoes are reported here for the first time.

The analysis of the effect of varying concentrations of the aqueous extracts of *Mentha spicata*, *Mentha piperita*, *Mentha pulegium*, and *Ocimum basilicum* on the mycelial growth of *Fusarium proliferatum*, *Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani* strains showed that the activity of these plants is manifested at high concentrations. Depending on the plant species, a variation in activity ranging from 0% to 100% inhibition of mycelial growth can be observed. This demonstrates that the observed antifungal effects vary depending on the dose. Furthermore, this confirms that the bioactive compounds of the plants are perceived as substances that produce effective effects against pathogenic fungi **(Probavathy et al., 2006).**

With the increase in the concentration of the aqueous extract of *Mentha spicata*, a potent antifungal activity and an increase in the antifungal index of mycelial growth against the tested strains were obtained. According to a study conducted by **Ullah et al. (2011)** it was demonstrated that the aqueous extracts of *Mentha spicata* collected from four districts in Northern Khyber Pakhtunkhwa have inhibitory activity against *Trichophyton longifusus*, *Candida albicans*, and *Aspergillus flavus*. According to **Alaklabi et al. (2016),** the aqueous extract of *Mentha spicata* root exhibits a remarkable antifungal response against *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, and *Microsporum audouinii*.

In our study, the aqueous extract of peppermint (*Mentha piperita*) exhibits significant antifungal activity against the tested strains. Research conducted by **Ilboudo et al. in 2016**

confirmed that the extract of *M. piperita* demonstrated antifungal activity on the fourth day of incubation against *Phoma sorghina* and *Fusarium moniliforme*, and this activity increases with the concentration of the extract.

The aqueous extract of *M. pulegium* also had an effect on the tested strains. **El Khetabi et al. (2023)** demonstrated that the aqueous extract of this plant in Morocco possesses antifungal activity against the growth of *Monilinia laxa* and *Monilinia fructigena* with inhibition percentages of 90% and 58%, respectively. According to the results of **Alharbi et al. (2021)** the methanolic extracts of *M. rotundifolia* and *M. pulegium* exhibited moderate antifungal activity against the yeast *Candida albicans* and two species of fungi (*Aspergillus flavus* and *Aspergillus niger*).

The aqueous extract of basil (*Ocimum basilicum*) caused inhibition of mycelial proliferation in the studied fungal strains. **In 2019, Nugroho et al**. demonstrated the efficacy of the aqueous extract of sweet basil against *Sclerotium rolfsii* in Los Baños, Philippines, with an inhibition rate of 33.35%. According to the research conducted by **Jacob et al. in 2016**, the aqueous extracts of *O. basilicum* showed growth inhibition against *Fusarium oxysporum*.

Salem et al. (2021) mentioned that methanolic extracts of basil exhibit potential antifungal properties against **Candida albicans**, with an inhibition diameter ranging from 30 to 35 mm in both cultivation countries (Tunisia and Egypt).

It is likely that this activity is caused by the nature and molecular structure of the active components in the aqueous extracts. These compounds traverse the cell membrane, penetrate the cell interior, interact with key intracellular sites such as enzymes and proteins, and induce cell death **(Omidbeygi et al., 2007)**.

The extracts obtained from the aerial parts of the four plants used in this study were subjected to phytochemical screening and revealed the presence of various secondary metabolites, including tannins, saponins, and alkaloids.

The antimicrobial activity is associated with the chemical composition of phenolic substances, whose structure (aromatic ring associated with hydroxyl group in various positions) allows them to form hydrogen bonds with the SH groups in the active sites of target enzymes, resulting in the deactivation of these enzymes in fungi **(Ultee et al., 2002; Cheikna et al., 2011**). Within this group of compounds, **Harborne and Williams (2000)** as well as **Sepúlveda et al. (2012)** assert that the detected tannins and flavonoids are known for their ability to inhibit the growth of numerous microorganisms, including bacteria and fungi. Moreover, phenolic compounds, terpenes, and steroids, which have been identified, are selected as essential oils, defending the plant against fungi and bacteria **(Raven et al., 2000).** Likewise, phenolic terpenes also act by binding to the amine and hydroxylamine groups of microbial cell membrane proteins, causing alterations in permeability and leakage of intracellular contents **(Lopez-Malo et al., 2005)**. Thus, the presence of the identified compounds and their biological properties constitute the scientific basis for the significant antifungal activity of the plants following the antifungal tests.

4. Conclusion

The obtained results are promising and confirm the potential use of aqueous extracts of *Mentha spicata*, *Mentha piperita*, *Mentha pulegium*, and *Ocimum basilicum* as antifungal agents in biotechnology, opening up possibilities for applications in agriculture as biofungicides.

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CONCLUSION GENERALE

Conclusion générale

La pomme de terre, culture vivrière, joue un rôle crucial dans la région d'El Oued, apportant une contribution significative à l'économie locale. La région est reconnue pour sa production de pomme de terre, constituant une source majeure de revenus pour les agriculteurs. En outre, elle participe à la diversification de l'économie agricole locale.

Malheureusement, les maladies fongiques constituent une menace majeure pour la production qualitative et quantitative de la pomme de terre à Oued Souf. Parmi les principales causes de perte de rendement figurent des pathogènes que nous avons pu identifier (1ère identification) tels que *Alternaria alternata* (responsable du chancre), *Wickerhamomyces anomalus* (causant le flétrissement), *Fusarium proliferatum* (provoquant la pourriture sèche) et *Rhizoctonia solani* (responsable de la galle noire). Ces maladies peuvent entraîner des pertes significatives en termes de rendement et de qualité des tubercules.

Afin de prévenir ces pertes, la lutte chimique reste une méthode cruciale pour gérer les maladies fongiques de la pomme de terre. Elle propose des solutions rapides et efficaces pour maîtriser les infections et réduire les pertes de récolte. Cependant, cette méthode comporte également des défis et des considérations importantes. L'utilisation intensive de fongicides chimiques peut entraîner des effets négatifs sur l'environnement, comme la contamination des sols et des eaux, ainsi que des impacts sur la biodiversité.

Actuellement, la lutte biologique représente une alternative prometteuse et durable à l'utilisation intensive de produits chimiques pour contrôler les maladies fongiques de la pomme de terre. Cette approche utilise des substances naturelles, comme les huiles essentielles et les extraits aqueux de plantes, pour réduire efficacement les infections fongiques. Notre choix s'est porté sur des plantes de la famille des Lamiacées, connue pour ses vertus médicinales.

L'analyse des huiles essentielles extraites par hydrodistillation des plantes étudiées, a montré que *Mentha piperita*, *Mentha pulegium* et *Mentha spicata* contiennent principalement du (-)-Carvone, tandis que le composé principal d'*Ocimum basilicum* est le linalol. Les résultats de cette recherche ont démontré que les huiles essentielles des plantes possèdent une activité antifongique significative contre les agents pathogènes de la pomme de terre, tels que *Alternaria alternata* et *Wickerhamomyces anomalus* les plus sensibles. Parmi les huiles essentielles

analysées, celles de *Mentha spicata* et de *Mentha piperita* qui se sont révélées les plus prometteuses, inhibant efficacement la croissance et la sporulation des champignons ciblés.

Les huiles essentielles de *Mentha spicata* montrent une efficacité antifongique complète (100%) à une concentration de 0,95% contre *Alternaria alternata* et *Wickerhamomyces anomalus*. De même, les huiles essentielles de *Mentha piperita* ont également abouti à une inhibition totale (100%) de la croissance mycélienne des souches testées, à une concentration de 0,87% pour *Wickerhamomyces anomalus* et de 0,90% pour *Alternaria alternata*.

L'analyse par HPLC a révélé que l'extrait aqueux des plantes étudiées contenait plusieurs composés chimiques différents, notamment l'acide acétylsalicylique, l'acide chlorogénique et l'acide *para*-coumarique. De plus, nous avons pu identifier la présence d'acide gallique, de caféine et de pyrogallol. Les résultats relatifs aux extraits aqueux testés, ont montré des effets anti-croissance évidents de *Mentha pulegium* et de *Mentha pepirita* sur les champignons étudiés, ainsi qu'un fort potentiel d'activité antifongique dont les plus sensibles sont *Alternaria alternata* et *Wickerhamomyces anomalus*. Pour *Mentha piperita*, l'extrait aqueux à 1% a entraîné une inhibition totale (100%) d'*Alternaria alternata* et de *Wickerhamomyces anomalus*. De plus, nous avons observé un effet fongicide inhibiteur complet (100%) à une concentration de 1,5% de l'extrait aqueux de *Mentha pulegium* contre *Alternaria alternata* et *Wickerhamomyces anomalus.*

Cette recherche a démontré le potentiel des extraits aqueux et des huiles essentielles comme agents antifongiques efficaces contre les champignons de la pomme de terre.

En perspectives, ces découvertes offrent une alternative durable et respectueuse de l'environnement aux méthodes de lutte conventionnelles, tout en contribuant à la sécurité alimentaire et à la préservation des ressources naturelles de la région. L'intégration de ces solutions dans les pratiques agricoles par l'utilisation de biofongicides, pourrait ainsi représenter une avancée majeure pour la protection des cultures et la promotion d'une agriculture plus durable.

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Annexes I

I-Classification botanique des plantes

I-1-*Mentha spicata*

Règne : Plantae

Embranchement : Spematophyta

Sous-embranchement : Anthophytina

Super-classe : Triaperturées

Classe : Triaperturées évoluées

Sous-classe : Asteridae

Super-ordre : Euastéridées I

Ordre : Lamiales

Famille : Lamiaceae

Genre : *Mentha*

Espèce : *Mentha spicata* **(Quezel et Santa, 1963)**

I-2-*Mentha piperita*

Règne Plantae

Sous règne Plantes vasculaires

Division : Magnoliophyta

Classe : Magnoliopsida

Ordre : Lamiales

Famille: Lamiaceae

Genre *Mentha*

Espèce *Mentha piperita* **(Paul, 1996).**

I-3-*Mentha pulegium*

Règne : Plantae

Embranchement : Phanérogames ou Spermaphytes

Sous-embranchement : Angiospermes

Classe : Eudicots

Sous-classe : Astéridées

Ordre : Lamiales

Famille : Lamiacées

Genre : *Mentha* (Tourn.) L.

Espèce: *Mentha pulegium L.*

I-4-*Ocimum basilicum*

Règne : Plantae

Sous règne : Plantes vasculaires

Embranchement: Spermatophytes

Sous Embranchement : Angiospermes

Classe : Dicotylédones

Sous Classe : Dialypétales

Ordre : Lamiales/labiales

Famille : Lamiaceae/labiaceae

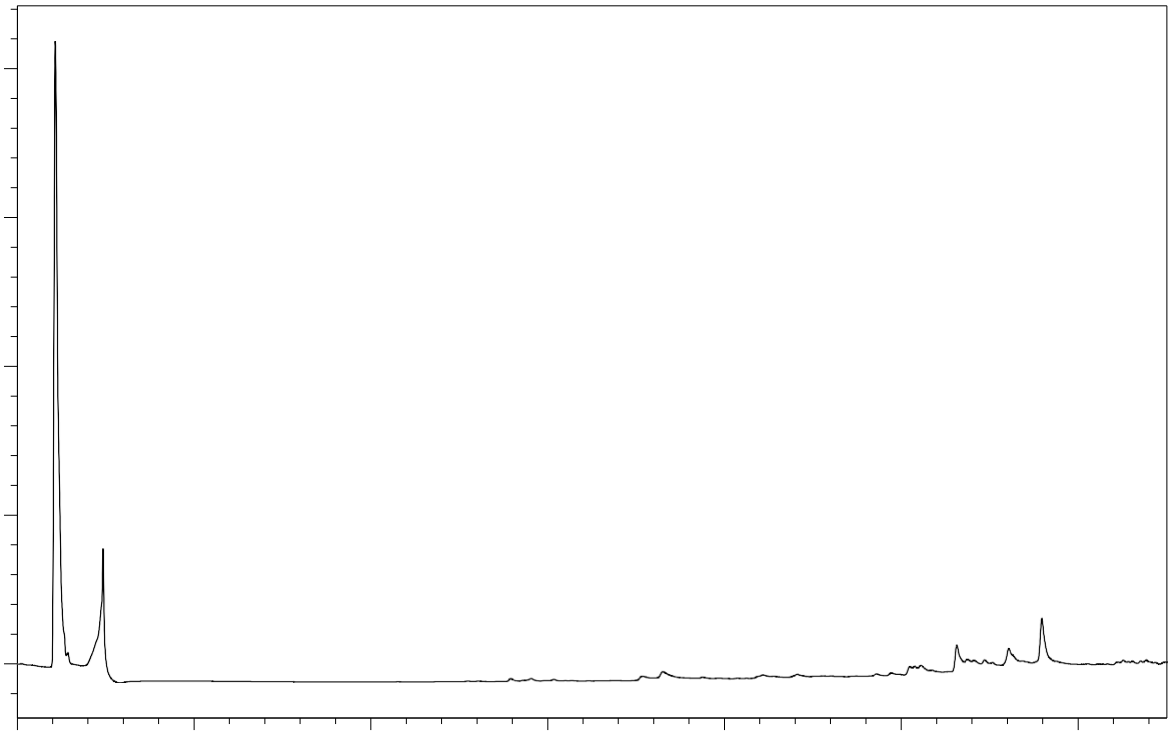
Genre : *Ocimum*

Espèce : *Ocimum basilicum* (**Angel *et al.*, 2012).**

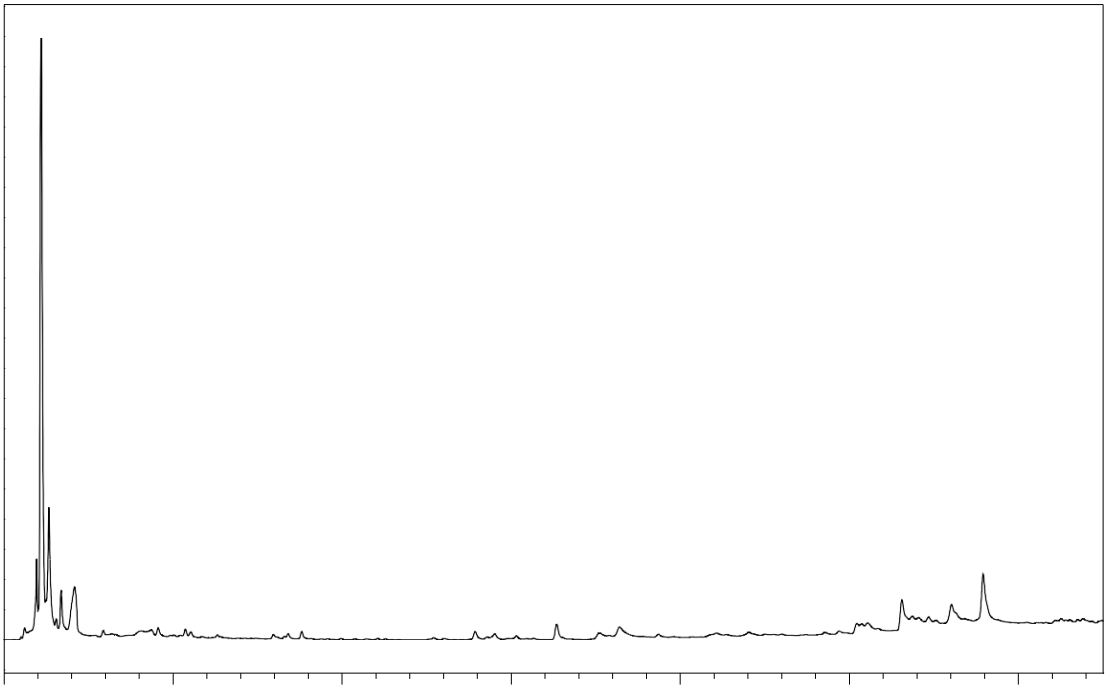
Annexes II

II-Chromatographie HPLC

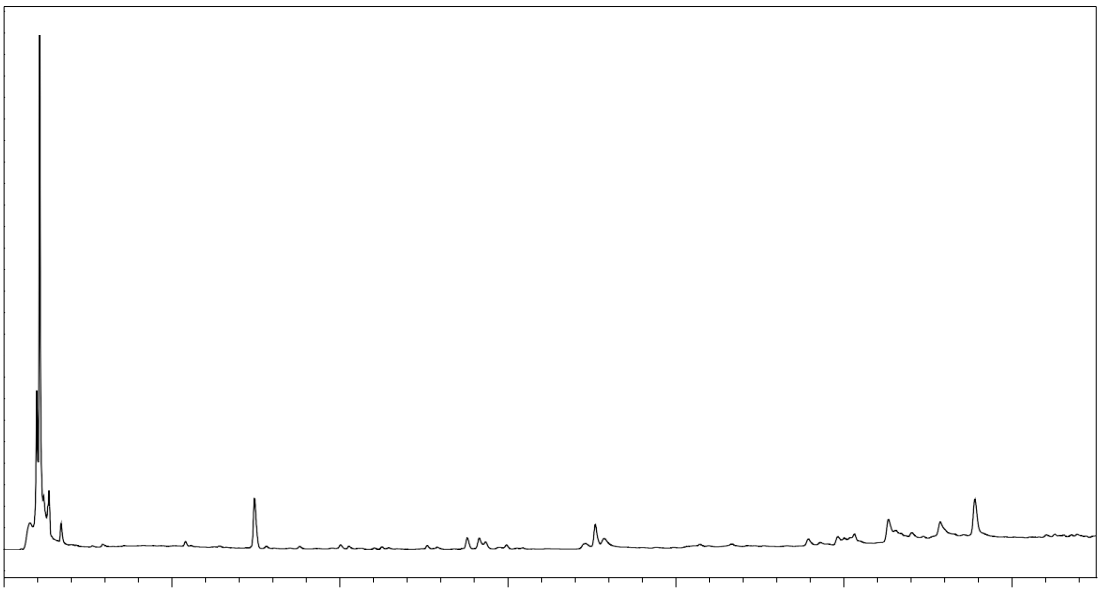
II-1-*Mentha spicata*



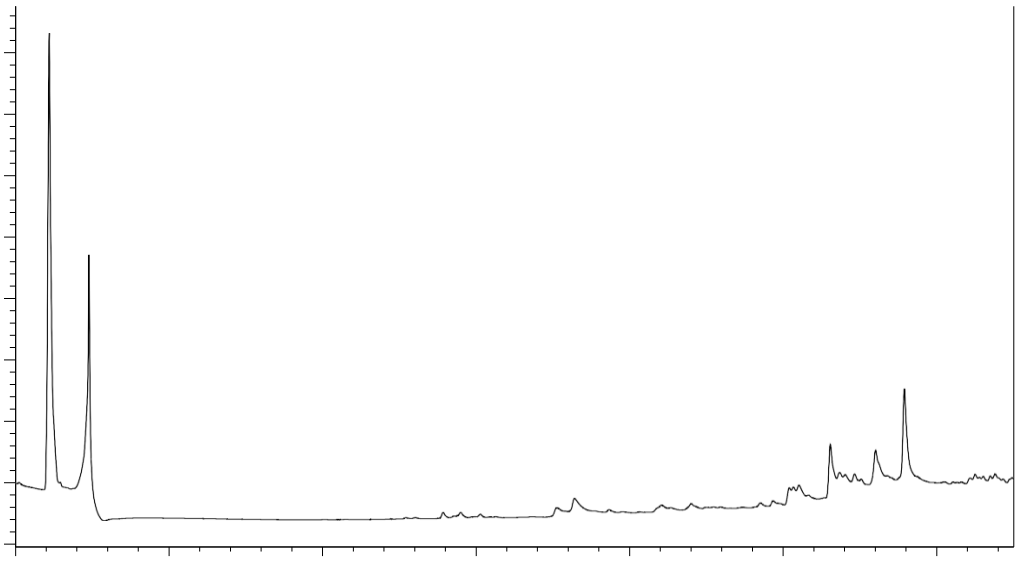
II-*2*-*Mentha piperita*



II-*3-Mentha pulegium*



II-*4-Ocimum basilicum*



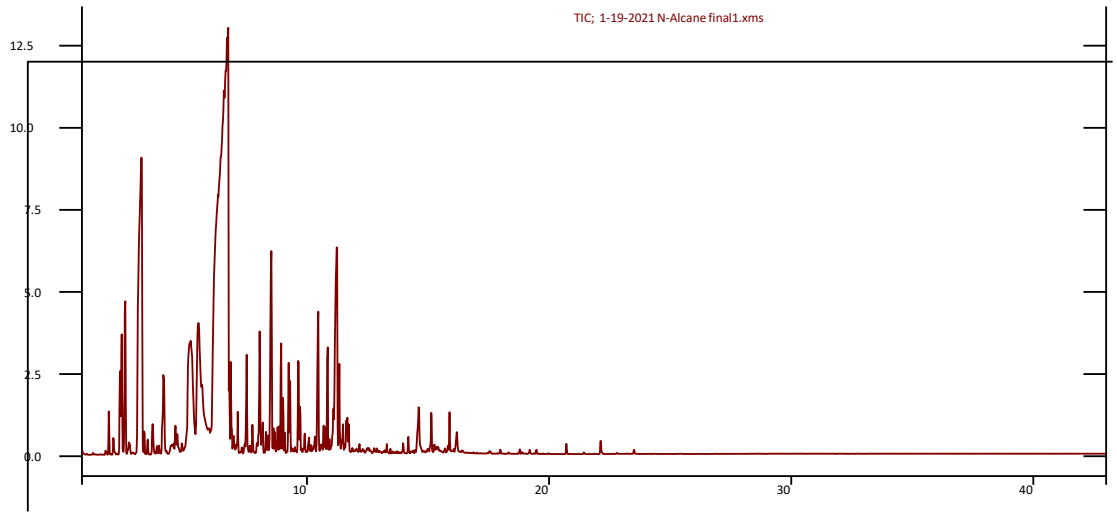
II-5-Standards

Title	Ret. Time	Height	Height%	Area	Area%
Acide acétylsalycilique.lcd	17.654	416790	100.000	3039110	100.000
Acide ascorpique.lcd	2.480	2546805	100.000	14316346	100.000
Acide caféique.lcd	11.072	3569518	100.000	24965945	100.000
Acide chlorogénique.lcd	8.771	892464	100.000	6915392	100.000
Acide gallique.lcd	3.770	3056772	100.000	16818861	100.000
Acide para comarique.lcd	14.243	1210894	100.000	8409610	100.000
Caféine.lcd	9.591	1740498	100.000	11357025	100.000
Catéchine.lcd	9.093	152244	100.000	1288924	100.000
Hipi catichine.lcd	9.084	155635	100.000	1308116	100.000
pyrogallol.lcd	5.352	205964	100.000	1773734	100.000
quercétine.lcd	21.296	4000236	100.000	37331942	100.000
Rutine.lcd	13.392	3997187	100.000	21642397	100.000
Vitamine B1.lcd	1.924	2854084	100.000	18876218	100.000
Vitamine B6.lcd	2.313	517530	100.000	1659645	100.000
Vitamine D3.lcd	2.473	169440	100.000	704028	100.000
vanilline.lcd	14.564	1336033	100.000	10636378	100.000

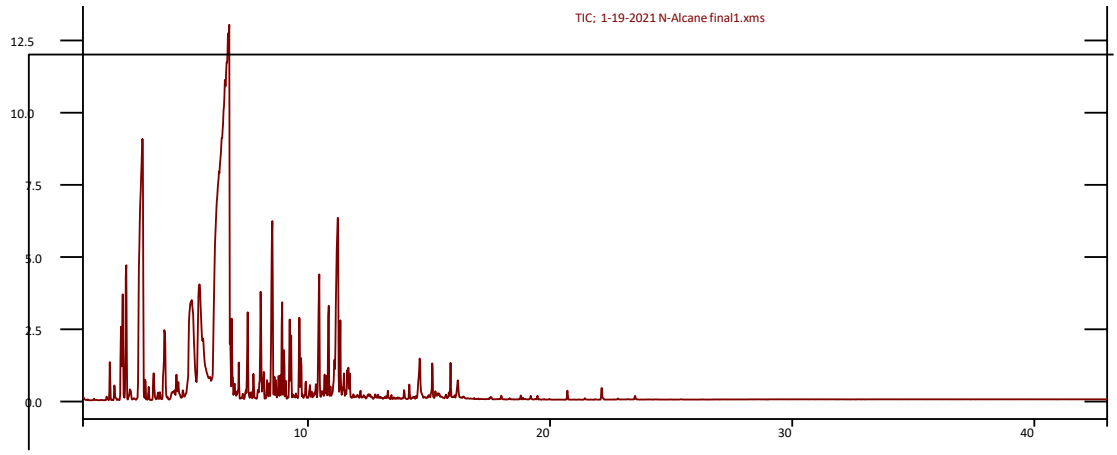
Annexes III

III-Détermination de la composition chimique des huiles essentielles par GS/MS

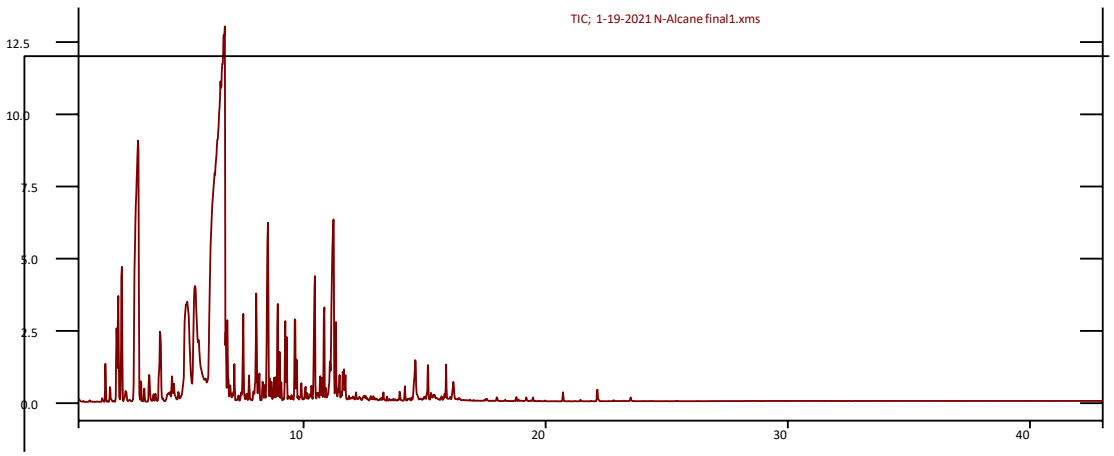
III-1- *Mentha spicata*



II-2-*Mentha piperita*



II-3-*Mentha pulegium*



II-4-*Ocimum basilicum*