الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria

وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research

جامعة الإخوة منتوري قسنطينة 1
Frères Mentouri Constantine 1 University
Université Frères Mentouri Constantine 1

Faculty of Natural and Life Sciences
Department of Microbiology

N° d'ordre :
N° de série :

Thesis submitted for the degree of the Master

Domain : Natural and Life Sciences
Sector : Biotechnology
Speciality : Mycology and Fungal Biotechnology

Entitled :

The antifungal activity of essential oils of *Thymus vulgaris* L. and *Rosmarinus officinalis* L. against *Alternaria alternata*

Presented by :
AISSAOUI Ikram
BERGUELLAH Nesrine
BOUTI Amani

Date of the defence : 20/06/2023

Jury members :

Supervisor: LABBANI Fatima-Zohra Kenza — MCA, ENS Assia Djebar - Constantine
Chair of the jury : LEGHLIMI Hind — MCA, UFM - Constantine
Examiner : BENOUCHANNE Djamila — MCB, ENSB - Constantine

Academic Year : 2022 - 2023

Dedication

The journey is over….it wasn't easy…..and no matter how long it took… it went through its good times and its bad times….and with the help of Allah , I complete this work and dedicate it to :

To my dear mother

To the light that illuminated my eyes, to the happiness of my existence, to a tender heart that illuminated my paths with its sincere prayers, my paradise, my mother "Saliha"

To my dear father

To whom I bear his name with pride, my support, the one who brought me to the highest rank, the light of my life, my father " Saleh "

My parents, this is your success, the success of the potion of trust, love and effort, this is the success of the fruit you planted and which is now blooming and radiating light.

To my dear husband

To my other half, the owner of my heart, and my husband who was my helper, who relieved me of all the burdens with which my days made me grow old, my hard rib "Younes Meddour", so to his mother "Zohra" and his father "Abd El-Hamid" rahimah allah.

To my sisters

To blood sisters and my cousins " Roumaissa, Hadil et Bassma" my friends thank you for your support, as long as you have endless strength.

To my two dear colleagues

To the partners of joys, shared together the trials of the road "Nesrine Berguellah" and " Amani Bouti"

To my supervisor

And the highest expressions of thanks and appreciation to our honorable teacher who worked hard and stayed awake to give our best " Fatima-Zohra Kenza Labbani"

Ikram Aissaoui

Dedication

***P**raise be to God for completing what I started, Here I am today wearing my hat, I don't know how to express my gratitude and thanks everyone who stood by me and helped me, Here I completed my career with many feelings inside me….*

***T**he journey wasn't short and it shouldn't be, the dream wasn't close and the road wasn't easy but I did it….*

I dedicate this graduation

***T**o the one whose name I carry with pride, that great man who brought out the best in me and always encouraged me to reach my ambition, to the one who reaped the thorns from my path to pave the path of knowledge for me…My dear father **Farid**, may God keep you as our shadow.*

***T**o the woman who made me an ambitious girl who loves challenges, my first role model from which I got to know strength and self-confidence, whose satisfaction creates success for me…My dear mother **Souad**, may God heal you and prolong your life with health and wellness.*

***T**o the one who has a wonderful touch in my life (distinguished) and gave me confidence and gave me a helping hand, to my support and the shoulder on which I always lean, to my life partner and the light of life, My fiancé and future husband **Ramzi**.*

***T**o My brothers, **Younes**, **Imad**, and to My sisters, **Zeyneb** and **Soumia**, the dear ones and the source of support in my life, and the blooming roses and fragrant winds, May nephews, may God protect them.*

***W**ithout forgetting the support, patience and understanding of My partners **Ikram** and **Amani** throughout this project, I wish you success in your lives and may your dreams come true.*

*And I would like to conclude the dedication with our honorable teacher"**Fatima-Zohra Kenza Labbani"** who helped us with her valuable advice, who made the hardships of the road easier for us and gave us better than we expected. I am grateful to you, Thank you very much, My God protect you for your family and your children.*

(Grateful to all of you, I would not have arrived without your grace upon me after God)

Nesrine Berguellah

Dedication

First of all, I would like to thank god for giving me the strength and the courage to carry out this modest work.

I would like to dedicate this humble work:

***To** my very dear parents **Azzedin and Hassiba** who accompany me with their prayers that God keep them for me.*

Thanks to their tender encouragement and their great sacrifices, they are able to create the affectionate climate conducive to the pursuit of my studies.

No dedication could express my respect, my consideration and my deep feelings towards them.

I pray the good Lord to bless them, to watch over them, hoping that they will always be proud of me.

***To** my dear brother **Mohamed Said,** who has always been an example of success for me.*

***To** my little brothers **Yakin** and **Youcef**, I wish them success in their lives.*

***To** my very dear and faithful friends **Maram, Sawsen, and Wafa**, with whom I have shared moments of joy and sadness.*

***To** my dear colleagues **Nesrine** and **Ikram** with whom I shared all the moments of fatigue and hardship on the road.*

***To** all those who have had an impact on my life and my success, to all those whom my heart has loved.*

***To** the professor supervising the research **"Fatima-Zohra Kenza Labbani"** thank you for your time and effort in evaluating our work and the valuable comments that you do not spare us.*

Amani Bouti

Thanks

First of all we would like to thank Allah, the merciful and the merciful for having given us the strength and the patience to carry out this modest work.

We would like to express my sincere thanks to our supervisor Professor "Fatima-Zohra Kenza Labbani" for the proposal of the theme and the follow-up of this work, for your precious advice during the period of the realization of this chapter of our lives, for your relevant remarks and your help, we are very honored by your support and to work with you.For all this, we would like to express our gratitude to her.

We would like to thank Doctor "Leghlimi Hind " who honored us with presiding over the jury for this soutenance.

We would like to thank Doctor "Benouchenne Djamila" who generously accepted to examine our work.

We would like to thank all the staff of the Department of Biotechnology.

Thank you for everything ……

Abstracts

Abstract

The present thesis aims to study the antifungal activity of essential oils (EOs) from the aromatic plants Thyme (*Thymus vulgaris* L.) and Rosemary (*Rosmarinus officinalis* L.) against the phytopathogenic fungus *Alternaria alternata.* This fungus is a ubiquitous phytopathogen capable of causing diseases to several agricultural crops and plants of economic interest.

In the present work, two research investigations based on the evaluation of the effectiveness of both *T. vulgaris* and *R. officinalis* EOs against the phytopathogenic fungus *A. alternata*, were studied. The antifungal power of the *T. vulgaris* and *R. officinalis* EOs was evaluated at concentrations of 50 and 100µg/ml on PDA medium, and the percentage of mycelial growth reduction was calculated. In the second investigation, the minimum inhibitory concentration (MIC) of both plant EOs was tested at different concentrations (from 0 to 12 µg/ml).

The obtained results from the first investigation showed that *T. vulgaris* EO showed the highest percentage of fungal growth reduction (53.4 and 74.9% at concentration of 50 and 100µg/ml, respectively) after 7 days of incubation. However, the results of MICdetermined in the second studied investigation, showed that *R. officinalis* EO was the most effective against the pathogen *A. alternata*, with a MIC value of 5000 µg/ml.

In conclusion, we can say that the studied EOs of *T. vulgaris* and *R. officinalis* have a strong inhibitory effect against the phytopathogenic fungus *A. alternata*. Hence, they can be considered as natural antifungal agents for controlling plant diseases caused by *A. alternata*.

Keywords: Phytopathogenic fungi, *Alternaria alternata*, Antifungal activity, Essential oils (EOs), *Thymus vulgaris*, *Rosmarinus officinalis*.

Résumé

Le présent mémoire a pour objectif l'étude de l'activité antifongique des huiles essentielles (HEs) des plantes aromatiques Thym (*Thymus vulgaris* L.) et Romarin (*Rosmarinus officinalis* L.) contre le champignon phytopathogène *Alternaria alternata*. Ce champignon est un phytopathogène ubiquiste capable de causer des maladies aux cultures agricoles et plantes d'intérêt économique. Dans le présent travail, deux recherches basées sur l'évaluation de l'efficacité des HEs de *T. vulgaris* et *R. officinalis* contre le champignon phytopathogène *A. alternata* ont été présentées. Le pouvoir antifongique des HEs de *T. vulgaris* et de *R. officinalis* a été évalué à des concentrations de 50 et 100 µg/ml sur un milieu solide PDA, et le pourcentage de réduction de la croissance mycélienne a été calculé. Dans la deuxième étude, la concentration minimale inhibitrice (CMI) des deux HEs a été testée à différentes concentrations (de 0 à 12 µg/ml). Les résultats de la première étude ont montré que l'HE de la plante *T. vulgaris* ont présenté le pourcentage de réduction de la croissance fongique le plus élevé (53.4 et 74.9% à des concentrations de 50 et 100 µg/100 ml, respectivement) après 7 jours d'incubation. Cependant, les résultats des CMI déterminées dans la deuxième étude ont montré que l'HE de la plante *R. officinalis* était la plus efficace contre le pathogène *A. alternata*, avec une valeur de CMI de 5000 µg/ml. En conclusion, nous pouvons dire que les HEs de *T. vulgaris* et de *R. officinalis* présentent un effet inhibiteur puissant contre le champignon phytopathogène *A. alternata*. Elles peuvent donc être considérées comme des agents antifongiques naturels pour lutter contre les maladies des plantes causées par le champignon *A. alternata*.

Mots-clés : Champignons phytopathogènes, *Alternaria alternata*, activité antifongique, huiles essentielles, *Thymus vulgaris*, *Rosmarinus officinalis*.

ملخص

يهدف عمل هذه المذكرة إلى تقديم دراسة حول النشاط المضاد للفطريات للزيوت الأساسية المستخلصة من النبتتين العطريتين، الزعتر (*Thymus vulgaris* L.) وإكليل الجبل (*Rosmarinus officinalis* L.)، ضد الفطر *Alternaria alternata* المسبب للأمراض النباتية. وهو نوع من الفطريات الموجودة في كل مكان وقادر على احداث أمراض للعديد من المحاصيل الزراعية والنباتات ذات الأهمية الاقتصادية. في هذا العمل، تم تقديم دراستين لبحثين علميين حول فعالية كل النبتتين العطريتين *T. vulgaris* و *R. officinalis* ضد الفطر المسبب للأمراض النباتية *A. alternata*. تم تقدير النشاط المضاد للفطريات لكل من *T. vulgaris* و *R. officinalis* بتركيزات 50 و 100 ميكروغرام/ مليلتر على الوسط المغذي الصلب PDA ، كما تم حساب نسبة انخفاض نمو الميسيليوم الفطري. في الدراسة الثانية، تم تقدير التركيز الأدنى المثبط (MIC) للزيوت الأساسية المستخلصة من النبتتين العطريتين باستخدام تراكيز مختلفة تتراوح من 0 إلى 12 ميكروغرام/ مليلتر. وقد بينت نتائج الدراسة الأولى أن الزيت الأساسي لنبتة *T. vulgaris*سمح بالحصول على أعلى نسبة من انخفاض نمو الميسيليوم الفطري (53.4 و74.9%، عند تراكيز 50 و100/ ميكروغرام/ مليلتر، على التوالي)، وذلك بعد 7 أيام من الحضن. أما بالنسبة للدراسة الثانية فقد أظهرت نتائج تقديرMIC أن الزيت الأساسي لنبتة *R. officinalis* كان أكثر فعالية ضد الفطر الممرض *A. alternata* ، بحيث كانت قيمة التركيز الأدنى المثبط 5000 ميكروغرام/مليلتر. في الختام، يمكننا أن نقول أن الزيوت الأساسية للنبتتين العطريتين *T. vulgaris* و *R. officinalis* لها تأثير مثبط قوي ضد فطر *A. alternata* المسبب للأمراض النباتي، وبالتالي يمكن اعتبارها كعوامل طبيعية مضادة للأمراض النباتية التي يسببها فطر *A. alternata*.

الكلمات المفتاحية: الفطريات المسببة للأمراض النباتية، *Alternaria alternata*، النشاط المضاد للفطريات، الزيوت الأساسية، *Thymus vulgaris*، *Rosmarinus officinalis*.

Table of Contents

Table of Contents

Chapter II : The fungus *Alternaria alternata*

Chapter III. Evaluation of the antifungal activity of Thyme and Rosemary EOs

Annexe

List of Figures

List of figures

List of Tables

List of tables

List of abbreviations

A. alternata :*Alternariaalternata*

A. tenuis :*Alternariatenuis*

A. solani:*Alternariasolani*

EB: Early blight

ABS:*Alternaria* brown spot

A. citri:*Alternariacitri*

ACT: Adenylate cyclase toxin

HST: Host-selective toxins

EOs: Essential oils

EO: Essential oil

T. vulgaris:*Thymus vulgaris*

R. officinalis: *Rosmarinus officinalis*

SFE: Supercritical Fluid Extraction

MAHD: Microwave-Assisted Hydro Distillation

SFME: Solvent Free Microwave Extraction

UAE: Ultrasound-Assisted Extraction

MHG: Microwave Hydro Diffusion and Gravity

TEOs: Thyme essential oils

TOE: Thyme essential oil

REOs: Rosemary essential oils

REO: Rosemary essential oil

A. oryzae: *Aspergillus oryzae*

A. brasiliensis :*Aspergillus brasiliensis*

A. flavus : *Aspergillus flavus*

A. parasiticus : *Aspergillus parasiticus*

A. ochracus : *Aspergillus ochracus*

EORO: Essential oil *Rosmarinus officinalis*

MIC: Minimum Inhibitory Concentration

MBC: Minimum Bactericidal Concentration

PDA: Potato Dextrose Agar

PDB: Potato Dextrose Broth

%: Percentage

µl: Microliter

Introduction

Introduction

The Mediterranean region is characterized by an exceptional biological diversity as well as considerable biological richness estimated at 25,000 species of vascular plants, which corresponds to 9.2% of the total diversity of species in a territory representing only 1.5% of the terrestrial surface. Algeria, due to its position, presents a great diversity of habitats occupied by an important floristic richness (Habib et al., 2020). According to Quézel and Santa (1962), the Algerian flora comprises 3139 species consisting of 3744 taxa including 464 endemic and 1818 more or less rare species.

Plants are natural biological factories. They produce active chemicals, such as essential oils (EOs), flavones, alkaloids, tannins, etc. and make them available to humans who can use them for their health and satisfy their vital needs (Croteau et al., 2000).

Rosemary *(Rosmarinus officinalis* L.) and Thyme *(Thymus vulgaris* L.) are one of the most important aromatic plants of the Lamiaceae family. EOs of *R. officinalis* L. (REOs) and *T. vulgaris* L. (TEOs) have been reported for their antifungal activities against different pathogenic fungi, such as *Aspergillus oryzae, A. niger, A. flavus,* and *Fusarium monilifirme* (Hammoudi et al., 2022).

Most EOs have antiseptic, antiviral, anti-infectious and anti-fungal properties (Belguendouz et Aboura., 2021). Moreover, the EOs of plants of the Lamiaceae family have generally interesting biological properties. The bioactive effects of their EOs and extracts have largely been shown to be related to their high richness in terpene and aromatic compounds whose chemical structures are much diversified (Merghache et al., 2016). They have numerous fields of application: in medicine.

However, to our knowledge, there are only a few investigations on the antifungal activity of REOs and TEOs against the phytopathogenic species *Alternaria alternata*, which is one of the most common pathogens found in a variety of natural food products including fruits and vegetables, cereal plants, seeds, and other plant organs (Barkai-Golan and Follett, 2017).

In this context, the present work report to study the antifungal activities ofthe EOs of two aromatic plants *R. officinalis* L. and *T. vulgaris* L. against the phytopathogenic fungus *A. Alternata.*

The present Master thesis contains three chapters. The first one includes generalities on the phytopathogenic fungus *A. alternata*. The second chapter contains an overview of the aromatic plants: *R. officinalis* L. and *T. vulgaris* L., as well as generalities on thyme and rosemary EOs.

The third and last chapter describes the evaluation of the antifungal activity of *T. vulgaris* and *R. officinalis* EOs, and brings out the obtained results and their discussion.

Chapter I
The aromatic plants, Thyme and Rosemary, And their essential oils

Chapter I
The aromatic plants,
Thyme and Rosemary,
And their essential oils

Chapter I. The aromatic plants, Thyme and Rosemary, and their essential oils

1. The aromatic plants, Thyme and Rosemary

1.1. Introduction

Aromatic and medicinal plants are the richest source of bioactive compounds (Purushothaman et Chandra, 2021). Algeria, with its large area and diversified climate has a varied flora. It is considered as one of the richest Arab countries with 3164 plants species (Benaiche, 2019).

Aromatic plants can be defined as those produce or exude volatile compound known as essential oils (EOs). An essential oil (EO) is a concentrated hydrophobic liquid with the tendency to vaporize easily, and dubbed 'essential' with reference to the fact that it captures the essence of a plant fragrance (Yoshinori, 2022). Aromatic plant have used for medicinal (bactericidal, fungicidal, virucidal, anti-parasitical, insecticidal) , cosmetic, agronomic, and sanitary application since ancient times (Birce et al., 2021). The difference between medicinal plants and aromatic plants, Medicinal plants also known as medicinal herbs, which can be defined as the plants that possess therapeutic properties or exert beneficial pharmacological effect on the human or animal body. Aromatic plants provide products with are extensively used as spices, flavoring agents and in perfumes and medicine. In addition, they also provide raw materials for the production of many important industrial chemicals (Monika, 2021).

One of the famous aromatic medicinal plant families in Algeria is the Lamiaceae Family (formerly called Labiatae). It contains about 236 genera and more than 7000 species (Bendif et al., 2020). It is a family of great diversity and variety with a cosmopolitan distribution. The species of this family are easily recognisable bysquare stems (four sided) and opposite leaves. The flowers are zygomorphic (rarely actinomorphic), usually bisexual and verticillaster (Carović-Stanko et al., 2016).

Most of the species belonging to the Lamiaceae family are important ornamental, medicinal, and aromatic plants, and produce essential oils. They have biological and medical application after confirmation by researches (Mamadalieva et al., 2017; Bendif et al., 2020). Some species of the Lamiaceae family are used as culinary herbs and grown for edible leaves, e. g basil (*Ocimum* spp.), mint (*Mentha piperita* L.), sage (*Salvia officinalis* L.), savory (*Saturja hortensis* L.), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.), lavender (*Lavandula angustifolia* Mill.), thyme (*Thymus vulgaris* L.), and rosemary (*Rosmarinus officinalis* L.) (Carović-Stanko et al., 2016).

Rosemary (*Rosmarinus officinalis* L.) and Thyme (*Thymus vulgaris* L.) are among the most common and popular aromatic plants in Algeria (Porte et al., 2007). They are mostly exploited for the extraction of EOs. Among the >3000 EOs known, about 300 are of importance for the pharmaceutical, agronomic especially to address the problems of agricultural crops(Neitoet al., 2017). The present part aims to provide an overview of the literature surrounding the description of aromatic plants rosemary and thyme of the family Lamiaceae.

1.2. Common thyme (*Thymus vulgaris* L.)

1.2.1. Generalities on thyme (*Thymus vulgaris* L.)

Thyme *(Thymus spp.)* is one of the most important genera of the *Lamiaceae* family *(*Moradi et al., 2023) with approximately 300–400 species (Figure 1) (Mancini et al., 2015; Faniand Kohanteb, 2017).The genus *Thymus* is a perennial medicinal aromatic plant wildly grown in different regions of the world. Mediterranean regions have been assumed as the origin of this plant (Sarfaraz et Rahimmalek, 2021). *It* is one of the most important edible plants, having many benefits. It is rich in phytonutrients, minerals, vitamins, flavonoids and antioxidants(Hammoudi Halat et al., 2022). The genus *Thymus* has numerous species and varieties and their EOs have been already studied. However, there are considerable research interests to continue with studying due to many other biological properties *Thymus* EOs may possess (Miloš Nikolić et al., 2014).

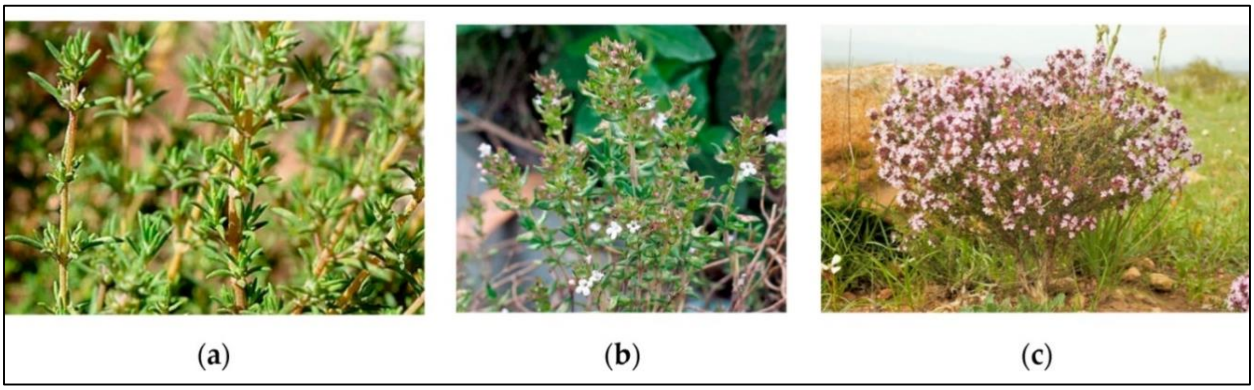

Figure 1. Some species of plants of the genus Thymus. (a): Thymus zygis subsp. Gracilis ; (b): Thymus vulgaris L.; (c): Thymus hyemalis (Nieto et al., 2020).

The common thyme (scientific name: *Thymus vulgaris L*.) is one of these evergreen aromatic plants, which is grown in various parts of the world for commercial purposes, mainly in the Mediterranean regions of Europe, North Africa, and Asia (Figure 1b) (Ferreira et al., 2016; Satyal et al., 2016; Fani et Kohanteb, 2017; Nieto, 2020; Silva et al., 2021). In recent years, the species T. vulgaris L. has gained popularity and become one of the most studied species of the genus Thymus (Taghouti et al., 2020; Patil et al., 2021).

1.2.2. Geographical distribution of *T. vulgaris* L.

1.2.2.1. In the world

T. vulgaris L., known as common thyme, is a perennial plant belongs to the genus *Thymus* and the Lamiaceae family (Hossain, et al., 2022). Native to Southern Europe, *T. vulgaris* L. has a worldwide distribution (Figure 2). It is indigenous to the Mediterranean region and other neighboring counties. It is also found in Northern Africa including Egypt and Saharan countries like Algeria, Morocco, Tunisia, and Libya. In addition, the plant is cultivated in Nigeria, Cameroon, and South Africa. It is also cultivated in European countries, such as Spain, France, Bulgaria, and Italy. Along the Mediterranean coastal region, it can be found growing up to 800 m from sea level. The plant can be cultivated by vegetative propagation using seeds, cuttings, or divided root sections (Patilet al., 2021).

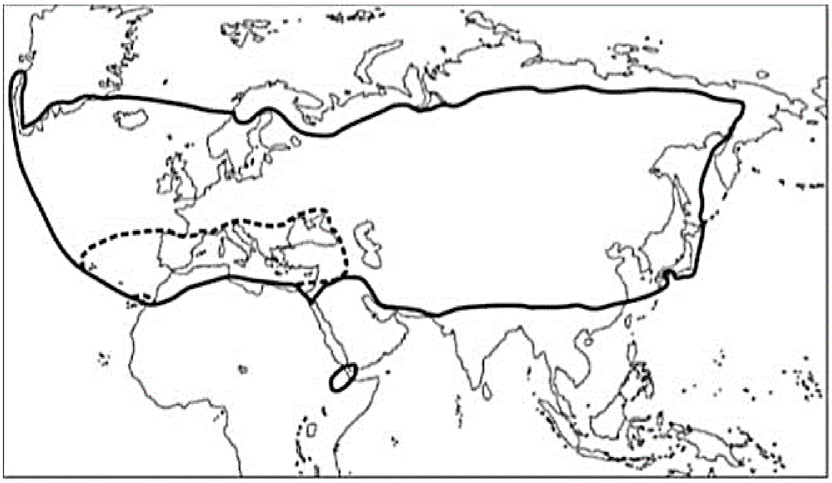

Figure 2. Geographical distribution of *T. vulgaris* L. in the world (Abdelli, 2017).

1.2.2.2. In Algeria

Thyme is represented by more than 300 species throughout the world, 12 of which are localized in Algeria and nine of them are endemic. These species are distributed along the national territory, from northern Algeria to Saharan Atlas, and from Constantine to Oran (Abdelli, 2017).

1.2.3. Botanical description

T. vulgaris L. is an aromatic, perennial, straight growing plant, which measures up 10-30 cm in height with woody base. Leaves are small, opposite, greyish green colored, oblong-lanceolate to linear, and are gland-dotted. They are measured up to 5-10 mm long and 0.8-2.5 mm wide with recurved margins. The flowers can be white, pink or purple, and are borne in whorls in early spring or summer depending on the conditions. The corolla is two-lipped and tubular, 6 to 8 mm long, and four pollen-bearing stamens protrude from the corolla. The nectaries are located at the base of the corolla (Obispo, 2022). The calyx is campanulate, covered by hairs and reddish glands (Patil et al., 2021; Cianfaglione et al., 2022).

The details of the different parts of *T. vulgaris* L. are shown in the Figure (3). However, the morphological characters may vary according to environmental condition. *T. vulgaris* L. grows well in arid, temperate, and unshaded areas. This plant grows well in hot, arid conditions with welldrained soil, and it is usually planted in the spring. The plant can be propagated using seed, cutting, or by dividing rooted sections. The plant also takes up deep freezes and can be found on mountain highlands (Patil et al., 2021).

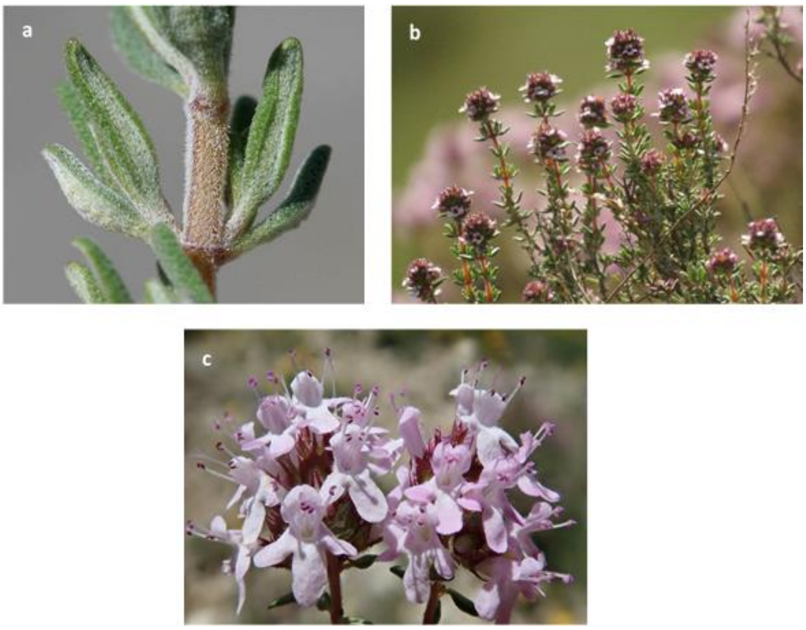

Figure 3. Morphological characterization of *T. vulgaris*. **(a):** Leaves of *T. vulgaris* (Paul busselen, 2023); **(b):** Flowering branches of *T. vulgaris* (Cianfaglione et al., 2022); **(c):** Complete flowersof *T. vulgaris* **(**Tinguy, 2023).

1.2.4. Taxonomic classification

The botanical situation of *T. vulgaris* species is given in the table (1).

Table 1. Classification of the common thyme (*Thymus vulgaris* L.) (Hammoudi Halat et al., 2022).

Taxonomic ranks	
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Seprmatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Subfamily	Nepetoideae
Genus	*Thymus*
Species	*Thymus vulgaris* L.

1.2.5. Chemical components

Several studies have reported that thyme is a rich source of bioactive compounds such as steroids, terpenoids, phenolic compounds, alkaloids, tannins, saponins, etc. (Nieto et al., 2020; Jain and Choudhary, 2022). *T. vulgaris* is characterized by chemical polymorphism according to the main volatile component (Galovičová et al., 2021).

T. vulgaris L. is one of the principal sources of phenolic compounds (Heidari et al., 2018). It is a rich source of flavonoids, such as apigenin, luteolin, cirsilineol, Sideritoflavone, xanthomicrol and thymosin (Figure 4a) (Jain and Choudhary, 2022).According to Roby et al. (2013), the methanolic fraction of *T. vulgaris* contains different phenolic acid like caffeic acid, cinnamic acid, p-coumaric acid, rosmarinic acid, ferulic acid, quinic acid (Figure 4b), as well as flavones and flavanones (Jain et Choudhary, 2022).

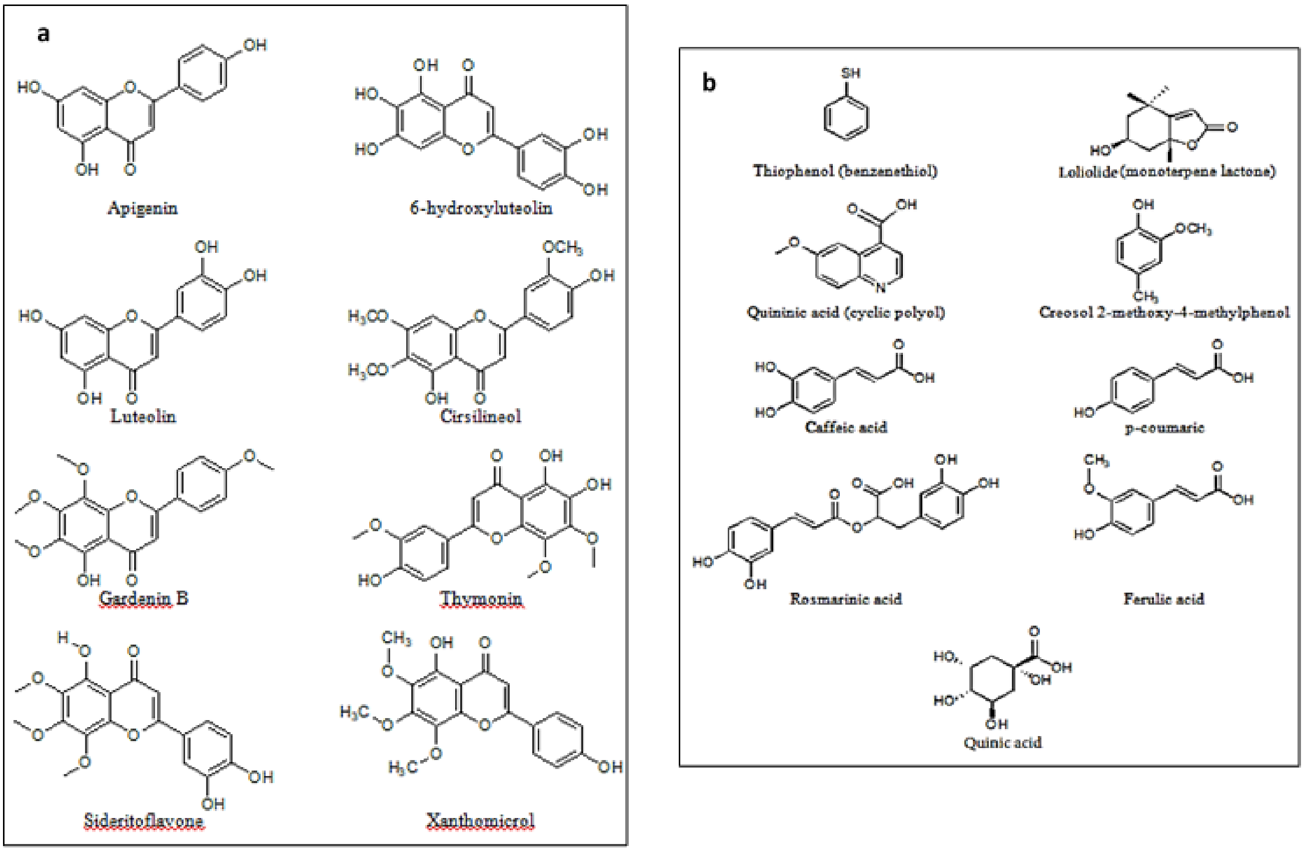

Figure 4. Some important compounds of *T. vulgaris*. (**a**): Major flavonoids of *T. vulgaris*; (**b**): Some phenolic acids of *T. vulgaris* (Jain et Choudhary, 2022).

Moreover, *T. vulgaris* is one of the major sources of monoterpene phenolic compounds like thymol and carvacrol (Jain et Choudhary, 2022). The latter is an isomer of thymol. Thymol provides thyme oil with its olfactory peculiarities. Depending o9455n the place of origin and the species of thyme, this oil offers percentages of phenolic content that range from 40 to 80% of thymol and up to 55% of carvacrol (Nieto et al., 2020).

More details will be provided in the third part of this chapter.

1.3. Rosemary (*Rosmarinus officinalis. L*)

1.3.1. Generalities

Rosemary is a highly aromatic, green, linear, evergreen perennial plant of the Lamiaceae family. Ithas a semi-creeping habit, very hardy with white flowers in autumn and at the end of winter (Medjher et al., 2021).

The genus *Rosmarinus* belongs to the mint family (Lamiaceae) together with oregano, thyme, basil and lavender (Datiles etAcevedo-Rodríguez, 2014; Pieracci et al., 2021). It is a small genus of evergreen shrubs with narrow, aromatic leaves and 2-lipped blue flowers borne in small clusters in the leaf axils. The genus name derives from the Greek *ros* and *marinus* (Dew of the sea), referring to its native seaside Mediterranean origin (Datiles et Acevedo-Rodríguez, 2014). The original habitat of the genus *Rosmarinus* includes the Mediterranean regions of Europe, Asia, and Africa. It grows from the sea level up to 1200 m above sea level (Sharifi-Rad et al., 2020). The Rosemary genus *Rosmarinus* is comprised of three species, namely *Rosmarinus tomentosus*, *R. eryocalix*, and *R. officinalis* (Figure 5) (Alu'datt et al., 2018; Saleh et al., 2022). Only the last two species are widely used in traditional medicine and as cooking ingredients (Pieracci et al., 2021).

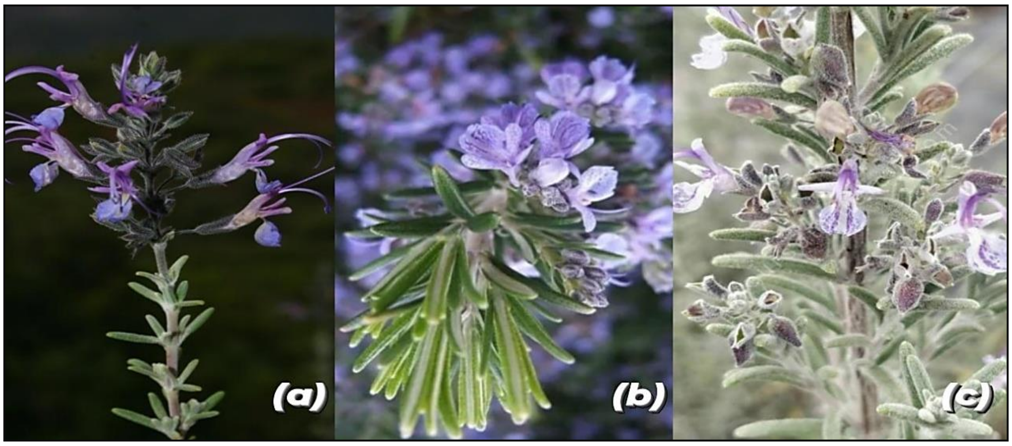

Figure 5. Some species of the rosemary genus *Rosmarinus.***(a) :***Rosmarinus eryocalix* (Vela, 2008) ; **(b) :***R. officinalis* (Kompelly, 2019) ; **(c) :***R. tomentosus* (Gorter, 2015).

R. officinalis L. (rosemary) is the type species of the genus *Rosmarinus*, as well as the most well-known rosemary species (Datiles et Acevedo-Rodríguez, 2014).It is a common and an endemic species to Algeria (Mehalaine et al., 2017), it is widely utilized for both culinary and therapeutic applications all over the world (Saleh et al., 2022).

Rosemary (*R. officinalis*) has been widely used since ancient time for herbal purposes and culinary uses, and it has been accepted as one of the plants rich in bioactive compounds with many phytotherapeutic activities. Potential bioactivities of rosemary plant are due to the many bioactive compounds that the species possess (Sharifi-Rad et al., 2020).

The extraction of aromatic plants, such as rosemary, has gained great interest in the last years (Sharifi-Rad et al., 2020). Regarding Andrade et al. (2018), this plant is of growing interest with an average number of 120 studies per year since 2010. Due to its applicationin pharmaceutical and food industries, the EO of *R. officinalis*has high commercial importance (Borges et al., 2018).

1.3.2. Geographical distribution

1.3.2.1. In the world

Rosemary has a very large geographical area. It is spontaneous throughout the Mediterranean region: Morocco, Algeria, Tunisia, Libya, France, Spain, Portugal, Italy, Greece, and Turkey (Outale, 2016). Although it has been introduced into many areas of the world since ancient times as an ornamental species (the rest of Europe, Chile, and China). However, it is in countries with a Mediterranean climate where it is most cultivated (Figure 6) (Francisco et al., 2020).

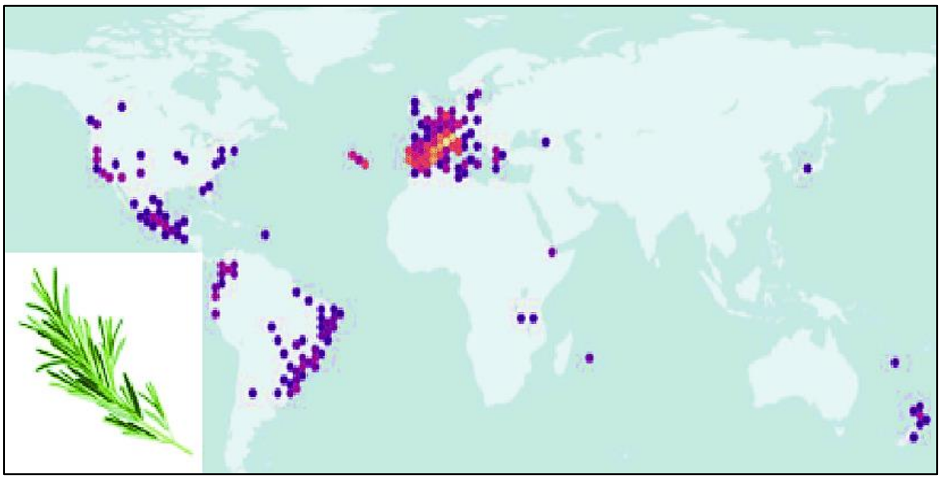

Figure 6. Geographic distribution of *R. officinalis* according to the Global Biodiversity Information Facility (GBIF) (Francisco et al., 2020).

In Figure (6), no marks are shown in China, Pakistan, India, South Africa, etc. This does not mean that rosemary is not found in these countries, but that it has not been registered in the Global Biodiversity Information Facility (GBIF) database. It lives between 0 and 1600 m above sea level and is indifferent to the type of soil. It does not grow well in very humid or very cold geographical areas. Its flowering period takes place between the end of December and April in the Mediterranean region (Northern hemisphere). Spain is the main producer of rosemary in the world for commercial purposes, followed by France, Italy, and Tunisia (Francisco et al., 2020).

1.3.2.2. In Algeria

This plant is quite apparent in different regions. It is found in the steppe at Sid Djilali in the region of Sid El Makhfi, so we can see it on the coast at Beni Saf in the Sidi Safi area located in the wilaya of Ain Temouchent. We can meet at different altitudes according to the bioclimatic stages, it is found in Tlemcen at lala Setti at 1025 meters (Mouas, 2018). In addition, rosemary is abundant in the municipality of Jijel. (Akroum et al., 2020).

1.3.3. Taxonomic classification

R. officinalis L. is one of the species in the genus *Rosmarinus* named by the naturalist and taxonomist Carl Linnaeus (Andrade et al., 2018). The table (2) shows the scientific classification of the species *R. officinalis* L.

Table 2. Scientific classification of *R. officinalis* L. (Andrade et al., 2018)

Taxonomic ranks	
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Seprmatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	*Rosmarinus*
Species	*Rosmarinus officinalis* L.

1.3.4. Botanical description

R. officinallis L. belongs to the Lamiaceae family, formerly called Labiatae. It is a shrub-type plant native of the Mediterranean (Figure 7) (Raphaelle et al., 2018). It is an aromatic evergreen plant with upright stems, whitish-blue flowers, and dark green leaves (Catarina et al., 2021), shurp up to 1.8 m tall, erect or procumbent. The leaves (10-41*1-3mm) are non-petiolate, of variable size in the same branch; they are strongly recurved and are sharpened, with a linear or lanceolate shape. The upper surface is green and the underside is whitish due to the presences of numerous hairs: glandular (capitate and peltate) and non-glandular trichomes. Its Flowers are small, arranged in short clusters, and they have a purple or white bilabiate corolla (8.5-13.5mm) (Gonzàlez-Minero et al., 2020).

This plant grows in dry or moderately humid soil, does not tolerate anaerobic or soaked soil and averagely tolerate salinity. Its flowering period often occurs between May and June in the

Mediterranean climate, and the fruiting period occurs between the spring and summer (Raphaelle et al., 2018)

Figure 7. (a): *Rosmarinus officinalis L.*(Mouffok, 2021).**(b):** Flowering branches of *Rosmarinus officinalis* (Svitone, 2021). **(c):** *Rosmarinus officinalis* (Kompelly, 2019).

3.5. Chemical components

The chemical composition of the plant as a whole depends on the place of growth and harvest as well as the time of harvest in the vegetative cycle. (Leplat , 2017). The composition of rosemary is variable between the extracts and the essential oil (Moumni, 2022). Regarding the extracts, the main bioactives present in Rosemary are carnosic acid, carnosol, rosmarinic acid, and ursolic acid. Figure (8) depict the structure of these bioactives, respectively. (Pappachan, 2023). The chemical components of the EOs will be shown in the third part of the present chapter

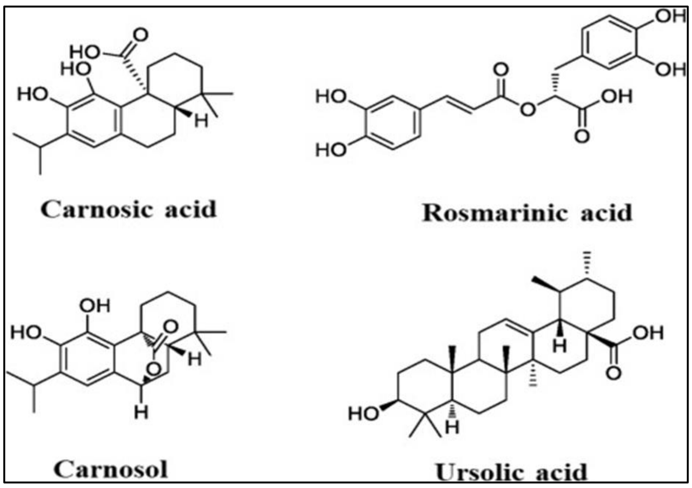

Figure 8. Important bioactive components in *Rosmarinus officinalis.L* (Pappachan, 2023).

1.4. Applications and uses of Thyme (*T. vulgaris* L.) and Rosemary (*R. officinalis*L.)

Medicinal and aromatic plants, which have a wide area of use, are used as a whole, fresh and dry. There are many different forms of use, such as leaves, roots, flowers, seeds, bark, tubers or aerial parts, which are also called herbaceous parts. Their fragmented or ground forms, and their extracts prepared in different ways (Dikme et al., 2023)

1.4.1. Applications and uses of thyme (*T. vulgaris* L.)

T. vulgaris has been used since ancient times to achieve healing, cure chest congestion, and induce saliva; the fresh leaves are taken to relieve sore throats. The plant is also used as an effective remedy for chest infections (bronchitis, pharyngitis, whooping cough) as well as to treat worms in children. The plant has been used for its flavor in cooking. *s of T. vulgaris* are used as an antiseptic, antiviral, and antimicrobial agent in folk medicine (Mandal et DebMandal, 2016).

In the other hand, thyme is used to feed marine animals and poultry (Rizwan et al., 2020). The use of thyme increases stability and reduces lipid oxidation during the shelf-lif period of foods (meat, meat products, milk, fish or fish products), which makes thyme a promising source of natural additives (Neito et al., 2020).

Today it is being used widely in nutraceutical, cosmeceuticals, as wellas pharmaceutical companies. Numerous researches have assessed the potential therapeutic benefits of thyme and showen effective results in treating nervous, respiratory, and cardiovascular-related disorders. However, it is beneficially used as antioxidant anticarcinogenic, antimicrobial, anti-inflammatory, antispasmodic, immunomodulator, and growth enhancer (Rizwan et al., 2020).

1.4.2. Applications and uses of *R. officinalis*L.

Rosemary (*R. officinalis*) is a plant with medicinal properties. Its extracts appear in the composition of hundreds of cosmetics. A Google search of the words "*Rosmarinus*" and "cosmetics" returns approximately 2,390,000 results in this search. It was observed that derivatives of rosemary are formulated in EOs for massages and aromatherapy, rosemary alcohol, gels, shampoos, soaps, rosemary water, cleansing milk, deodorant, anti-wrinkle cream, aftershave lotion, hydrating facial cream, cream for the eye contour area, etc. (Francisco, 2020).

R. officinalis L. has been also used in folk medicine to alleviate several diseases including headache, dysmenorrhea, stomachache, epilepsy, rheumatic pain, spasms, nervous agitation, improvement of memory, hysteria, depression, as well as physical and mental fatigue (Rahbardar, 2020).

2. Essential oils (EOs) of Thyme and Rosemary

2.1. Generalities on essential oils (EOs)

2.1.1. General definition

EOs are natural, volatile and aromatic liquids extracted from special plants. They are a complex mixture of secondary metabolites (terpenes, phenolic compounds, alcohol) (Falleh et al., 2020). EOs represent 5% of the vegetal dry matter, and could be obtained from roots, leaves, bark, branches, flowers, fruits, and seeds (Baptista-Silva et al., 2020). All of these organs of aromatic plants are utilized to distill EOs, for example, seeds (Caraway, Cumin, and Coriander), leaves (Mint, Thyme, Rosemary, Sage, Oregano, Basil, Celery, and Parsley), fruits (Anise, Fennel, and Lemon), flowers (Rose and Rosemary), bark (Cinnamon), cloves or buds (Clove and Garlic), and rhizomes (Ginger) (Mohamed et al., 2022).

The volatile compounds present in EOs have an important environmental function as they can protect plants from invasive bacteria, fungi, insects, and viruses. They are also able to attract certain insects for plant pollination (Mohamed et al., 2022).

The chemical composition of EOs is determined by genetic factors (Freitas de Sa Filho, 2022). However, other factors can lead to significant changes in the production of these constituents, such as geographical location, climatic elements (temperature, precipitation, solar irradiation), and stage of maturity (Freitas de Sa Filho, 2022; Mohamed et al., 2022).

2.1.2. Location and place of synthesis

EOs are located in all living parts of the plant, and they areform in the cytoplasm of variable secretory cells depending on the plant organconsidered (Mohamed et al., 2022). Then, they usually accumulate in specialized glandular cellscovered with a cuticle. Subsequently, they are stored in structuresspecialized histological features, often located on or near the surface of the plant, namely EOs cells: epidermal glandular hairs that produceso-called superficial species (Families of Labiaceae, Geraniaceae, and Rutaceae), secretory pockets(Families of Myrtaceae, Auranthiaceae, and Rutaceae) or secretory ducts (Families of Apiaceae,Ombelliferaceae, and Asteraceae). EOs can be extracted from various organsof the plant (Mihoub et al., 2019).

2.2. Extraction methods of EOs

EOs have been used by many cultures around the world for centuries for different purposes according to each culture (Kumar et al., 2022). The extraction is a crucial and fundamental step in numerous processes, especially in extracting EOs and oleoresins for applications, such as flavours and fragrances. They are further isolated as bioactives used in cosmeceuticals, functional foods, therapeutics and healthcare (Setgunga et al., 2022).

EOs can be extracted from several plants with different parts by various extraction methods (Kumar et al., 2022). Naturally produced by aromatic plants and commonly obtained by hydrodistillation or steam distillation, EOs are synthetized by all aromatic plant organs, flowers, buds, leaves, seeds, fruits, roots and rhizomes, wood and bark in relatively small amounts (Raveauand al ., 2020). The oil extraction techniques in plants are further classified into traditional and modern techniques (KaurVirkPannu et al., 2018).

2.2.1. Traditional techniques

2.2.1.1. Hydrodistillation

Hydrodistillation is the oldest and simplest oils extraction method, which was discovered by Avicenna. Rose was the first plant extract used and purified by this method (Aziz et al., 2018).
Hydrodistilation is considered as a unique method to extract plant materials like wood or flower, and is frequently used for extractions involving hydrophobic natural plant material with a high bioling point. As the oils are surrounded by water, this method is able to protect EOs to be extracted at a certain degree without being overheated. The main advantage of this extraction technique is its ability to isolate plant materials below 100°C (Aziz et al., 2018).

The principale is based upon isotropic distillation at atmospheric pressure and heating (during extraction process), water, oil, molecules and other solvents (KaurVirkPannu et al., 2018). The procedures start with immersing the plantmaterials directly into water inside the vessel, and whole mixturewasboiled. The devices include a heating source, vessel, a condenser to convert vapor from vessel onto liquid, and a decanter to collect the condensate and to separate EOs with water (Kumar et al., 2022). The following figure (9) decribes hydrodistillation of EOs (Ghasemy-piranloo et al., 2020).

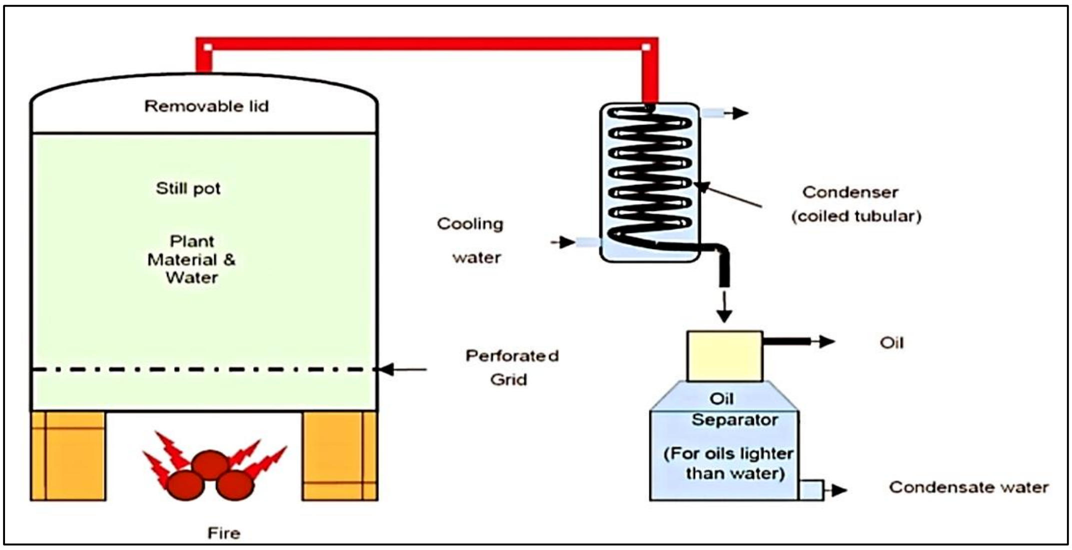

Figure 9. Hydrodistillation of EOs (Ghasemy-piranloo et al., 2020).

2.2.1.2. Steam distillation

Altough EOs are produced by different methods, the majority (93%) are produced by steam distillation (Almeida-Couto et al., 2022). Steam distillation is the oldest and the traditional method of oil extraction (Herman et al., 2019). This method is a standard method used for temperature-sensitive materials (such as oil, resin, hydrocarbon, and many others), insoluble in water, and can decompose at its boiling point (Immaroh et al., 2021). It is used for temperature sensible plants like aromatic compounds. It was used for purifying organic compounds, but still an important method in industriel sectors (KaurVirkPannu et al., 2018).

The percentage of EOs being extracted by this technique is 93% and the remaining 7% can be further extracted by other methods. Basically, the process starts by heating of plant material using steam which is supplied from steam generator. Heat is the main factor determining how effectively the plant material structures break down, burst, and release the aromatic components or EOs (Kumar et al., 2022).

Masango (2005) developed an innovative steam distillation extraction technique to increase the isolated EO yields and reduce the amont of wastewater produced during the extraction process. The system uses a packed bed of the plant samples, placed above the steam source. Only steam is allowed to pass through the plants and boiling water does not mix with the botanical materials (Figure 10). Therefore, the process requires less steam and the amount of water in the distillate can be reduce (Aziz et al., 2018).

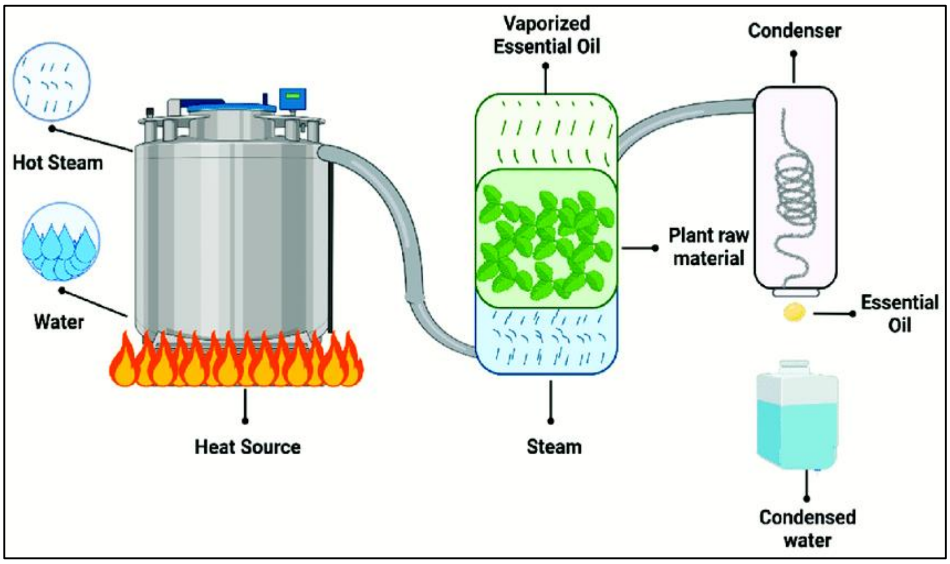

Figure 10. Steam distillation of EOs (Machado et al., 2022).

2.2.1.3. Solvent extraction

Solvent extraction, also called liquid-liquid extraction, can be used to separate a solute from a solution by extraction into another solvent. The two solvents must be essentially immiscible.

(Tawler et Sinnott, 2013). It is one of the most commonly applied conventional separation techniques (Rehab , 2021).

Solvent extraction can be used to extract EOs that are thermolabiles (example: from blossom). During this method, the plant material is placed into a solvent bath, which dissolves it. After the extraction, the liquid mixture that contains the EO (along with other compounds) goes through a filtration process and a subsequent distillation. (Stratakos et Koidis , 2016).

The filtrate contains a resin (resinoid), or the mixture of wax, fragrance, and essential oil. Alcohol is combined with the filtrate mixture in order to dissolve the essential oil into it and thereafter distilled at low temperature. During the distillation process, the alcohol absorbs fragrance and is evaporated while the aromatic absolute oil remains in the pot residue. (Kumar, 2022).

Solvents that are commonly used for extraction are hexane, ethanol, petroleum ether, and methanol. The main advantage of extraction over distillation is that a lower temperature is used during the process, therefore reducing the risk of chemical changes due to high temperatures, which are used during distillation. Solvent extraction is inexpensive and relatively fast and because the diffusion rates are influenced by temperature, it is possible to increase the speed of the process by using hot solvents. The EOs produced will contain a small quantity of solvent as a residue, and therefore its use for food applications is not possible. However, if the solvent used is alcohol like ethanol , it is safe for consumption and considered “food grade” . (Stratakos et Koidis, 2016).

This method is used in different industries including food, pharmaceutical, petrochemical, ore processing, and waste management to extract beneficial components (Rehab, 2021).

2.2.2. Modern techniques

The modern oil extraction techniques in the plants, includes: Supercritical Fluid Extraction (SFE), Microwave-Assisted Hydro Distillation (MAHD), Solvent Free Microwave Extraction (SFME), Ultrasound-Assisted Extraction (UAE), Microwave Hydro Diffusion and Gravity (MHG), and other modern oil extraction methods for EOs(Figure 19)(KaurVirkPannu et al., 2018).

2.3. Chemical components

2.3.1. Chemical components of thyme (*T. vulgaris*)EOs

The main compounds of the *T. vulgaris* EOs (TEOs) are terpenoids and phenolic derivates, The important components of TEOs are thymol, γ-terpinene, p-cymene, carvacrol, eugenol,camphor, linalool, β-myrcene, camphene, borneol, limonene, α-thujene (Aljabeili and al., 2018; Jain and Choudhary, 2022). In addition, Galovičová et al. (2021) showed that the main components of *TEOs* were thymol, *p*-cymene, 1,8-cineole (6.7), γ-terpinene, and carvacrol. TEOs contains also α-terpineol, and caryophyllene (Hossain et al., 2022).The figure (11) showed the chemical structure of the main components of *TEOs*.

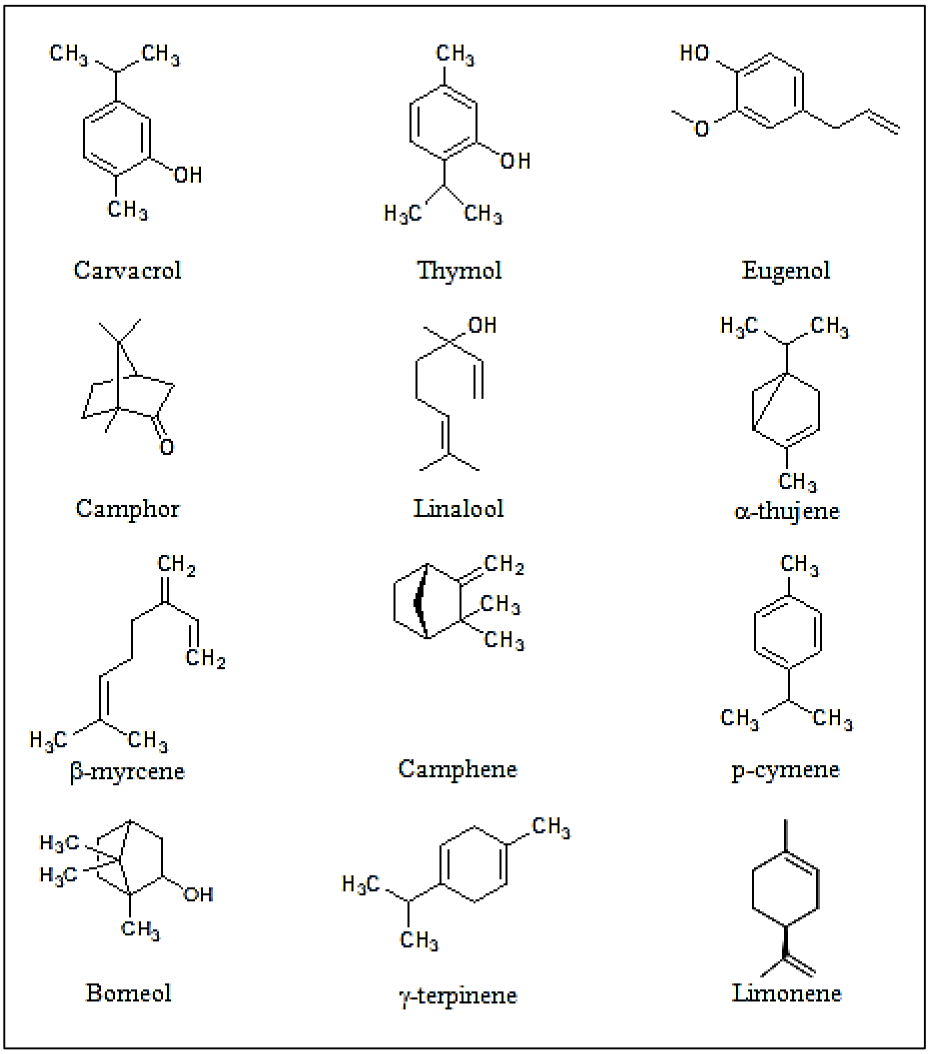

Figure 11. Some important EOs components of T. vulgaris *(*Jain et Choudhary, 2022).

2.3.2. Chemical components of rosemary (*R. officinalis* L.) EOs

Previously reported studies about the chemical composition of REOs showed certain diversity in chemical profile and composition.

The obtained results of Micic et al. (2021) showed that three compounds were principle in REOs: α-pinene (17.76 - 23.00%), eucalyptol (17.79 - 23.40%), and camphor (14.39 - 17.17%). Pellegrini et al. (2018) found camphor to be the principal compound (22.07%) followed by α-pinene (16.64%), eucalyptol (15.71%), and borneol (11.99%). Similar results were obtained by Zaouali et al. (2010) but with eucalyptol as the principal compound. Jordan et al. (2013)investigated also the chemical components of REO, and the major compounds were α-pinene (13.0–15.5%), eucalyptol (18.9–21.2%), and camphor (17.0–18.6%) (Micic et al., 2021). In addition, the results of Verma et al. (2019) showed that the major constituents of the REO were camphor (23.9–35.8%), 1,8-cineole

(18.0–23.9%), α-pinene (4.5–14.4%), verbenone (6.5–12.4%), camphene (2.5–6.9%), limonene (2.1–2.8%), bornyl acetate (1.1–4.1%), α-terpineol (1.9–3.6%) and β-pinene (2.1–3.3%).

These results demonstrate that REO are composed of terpenes, whiche have medicinal properties and biological acitivity. The figure (12) showed the chemical structure of the main rosemary EOs compounds.

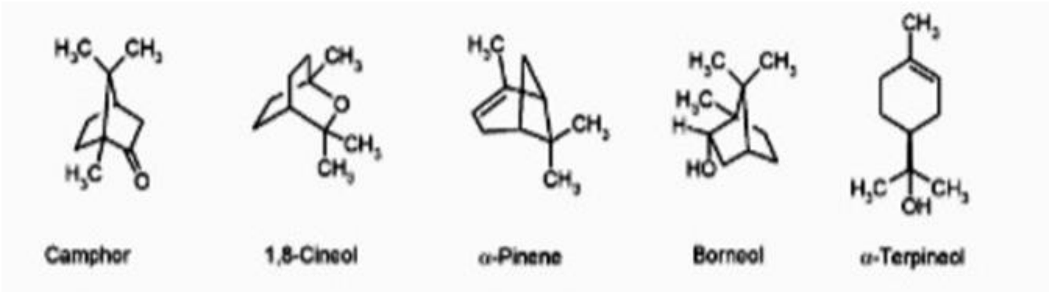

Figure 12. Chemical structures of the main compounds of rosemary essential oils. (Wollinger,2016).

2.4. Biological properties

2.4.1. Biological properties of thyme (*T. vulgaris*) EOs

2.4.1.1. Anti-inflammatory activity

TEO is a combination of monoterpenes. The most compounds of this oil are the natural terpenoid thymol and its phenol chemical carvacrol that has medicinal drug, anti-oxidative, antimicrobial, anti-tissue, anti-spasmodic, and antibacterial effects (Hosseinzadeh et al., 2015).

Thyme has been traditionally used as an herbal medicine for inflammatory diseases, and serval investigations showed that it contains valuable components with anti-inflammatory properties. The collected *T. vulgaris* from Brazil, Italy and Greece have been shown to be effective against inflammation. Moreover, *thymus* extracts and EOs have shown to have a potent attenuating effect for several inflammatory mediators (Lorenzo et al., 2018).

2.4.1.2. Anti-microbial activity

Plants from the genus *Thymus* are important medicinal herbs, which are known to contain antimicrobial agents, and are rich in different active substances (Kuete et al., 2017).The EO of *T. vulgaris* L. and plant-basedthymol are characterized by strong antimicrobial potential and can be utilized as bio-preservative agents in meat products (Posgay et al., 2022).

The antimicrobial activity of thyme depends on their chemical constituents especially TEO (Borugăet al., 2014). EOs that are rich in phenolic compounds appear to be the most effective compounds against infections caused by microorganisms. As reported above, TEO is a “natural” preservative with the ability to control microorganisms (Nieto et al.,2020).*T. vulgaris* L. proved to be promising source of bioactive substances (Gedikoglu, 2022) including EO and thymol, which can prevent the growth and spread of undesirable microorganisms. However, their antimicrobial mechanisms at the molecular and cellular levels are still not fully under-stood and only a few EO-based, commercially manufactured food preservatives are available today (Oliveira et al., 2020; Pandey et al., 2021).

Although the *in vitro* antimicrobial activity of *T.vulgaris* leaves EO on some human pathogens are widely documented, the effects of this oil on oral pathogens such as periodontopathic and cariogenic microorganisms are not fully understood (Fani et al., 2017).

- **Antibacterial and antiviral activities**

The EO obtained from *T. vulgaris* harvested 4 biological process stages were evaluated for their biological activity and chemical components. The thyme volatile oils were analyzed for their inhibition effects against 9 strains of gram-negative bacteria and 6 strains of gram-positive bacteria (Hosseinzadeh et al., 2015). The oil from thyme fully flower was the foremost effective at stopping the growth of the microorganism species examined. The oils tested were conjointly shown to possess smart antibacterial activity by direct contact, that gave the impression to be a lot of marked against the gram-negative microorganism. Some species were capable of recovering a minimum of 50% of their metabolic function once contact with the inhibitor, whereas most of the strains were shown to have been inactivated almost completely (Dauquan et Abdullah, 2017).

Recently, and with the coronavirus disease, COVID-19, perplexing healthcare systems and societies, intensive efforts to develop effective preparations against severe acute respiratory syndrome coronavirud-2 (Sars-CoV-2) were prominent, and natural compounds were not an exception. TEO has previously shown effectiveness against several RNA viruses including humans and feline coronaviruses (Hammoudi Halat et al., 2022).

- **Antifungal activity**

The antifungal activity of thyme is mainly contributed to the phenolic compounds, thymol and carvacrol. The latter was reported more than two decades ago, to have potent fungitoxic activity when tested on fruits to inhibit the growth of fungi. Regarding the antifungal capacity, findings of the different studies are not always comparable, due to variances in herb quality, qualitative and quantitative variations in EO constituents, and differences between fungal strains examined, and methodological differences (Hammoudi Halat et al., 2022).

TEO was reported for its antifungal effects against food spoilage fungi, including *Aspergillus* species, such as *A. oryzae*, *A. brasiliensis*, *A. flavus*, *A. parasiticus*, *A. ochracus*, and *Fusarium monilifirme*. EOs from two clonal types of *T. vulgaris* (Laval-1 and Laval-2) also displayed antifungal activities against tow common storage pathogens, *Botrytis cinerea* and *Rhizopus stolonifer* (Kuete, 2017). These EOs exhibited antifungal activity through apoptosis, nuclear condensation, and plasma membrane damage. Additionally, the oil decreased aflatoxin production and gene expression and adversely affected secondary metabolism and mechanisms of virulence (Hammoudi Halat et al., 2022).

The thyme oil contains thymol, and 1,8-cineole as a major component and three times stronger inhibition as thyme oil exhibited by pure thymol. The antifungal activity of thyme oil was reported against *A. alternata*. This oil is extracted from the leaves of *T. vulgaris* was used as a potential agent to protect several food commodities from microbial deterioration caused by food pathogenic fungi. Moreover, the TEO showed significant fungitoxic activity against *A. alternata*, and inhibited the mycelial growth of the fungi ultimately increasing the shelf life of fruit for consumption. The use of EO as an antifungal agent can be an interesting and promising approach for the management of postharvest diseases of fruit and vegetables. The TEO with its strong fungitoxic effect, non-hazardous, high inhibitory action, and the increase in fruit shelf life, which are the desired characters of an ideal fungicide and can be recommended as a botanical fungitoxicant (Aslam, 2022).

2.4.2.Biological properties of rosemary (*R. officinalis* L.)EOs

Rosemary has played an important role from past to present and has antimicrobial, antifungal and antioxidant properties. With these features, it is used in many sectors, especially food and pharmacy. REOs have positive effects on biological activity (Erkan et al., 2020). *R. officinalis* L.exhibits many biological activities that have been clinically proven, including antimicrobial, antiviral, antiproliferative, anti-inflammatory, and antioxidant activity (Patel et al., 2023).

2.4.2.1. Antioxidant activity

The antioxidant activity of the OEs of *R. officinalis* L. was compared with that of three of its main components (1,8-cineole, a-pinene, b-pinene). In general, REO showed greater activity than its components taken isolation. The antioxidant activities of all the samples tested were mainly dose-dependent. Hence, the REO presents potential application as a natural preservative in the food industry, thanks to its ability to act as free radical scavengers (Leplat, 2017).

REO, besides exhibiting free radical scavenging activity, mediates its hepatoprotective effects also through activation of physiological defense mechanisms. Natural antioxidant products are increasingly being used to treat various pathological liver conditions considering the role of oxidative stress in their pathogenesis (Rašković et al., 2014; Marion, 2017).

2. 4.2.2. Anti-inflammatory activity

Among EORO biological activities, its anti-inflammatory capacity stands out. It is important to notice that other monoterpenes besides 1,8-cineole, α-pinene, and camphor may also contribute to this property of EORO_like limonene and myrcene (Borges et al., 2019).

Faria et al., (2011) tetested EORO (doses ranging from 100 to 1000 mg/kg, p.o) in rat models of carrageenan-induced paw edema. Interestingly, in this study, the treatment with EORO had 64% less gastric damage compared to the group treated with indomethacin; this is highly relevant because the continuous use of non-steroidal anti-inflammatory drugs leads to gastric damage and peptic ulcer (Drini, 2017). Faria et al., (2011) and inhibited 50%of edema formation; the latter was administered at 498 μg/kg and inhibited 46% of edema formation. That is highly relevant, considering that similar results were obtained with a 60 times smaller dose of EORO when administered as a nano-emulsion. Borges et al. (2018), further studied this same dose of EORO nano-emulsion in carrageenan-induced abdominal edema in zebrafish; compared to the non-treated group, the treatment with EORO's nano-emulsion inhibited 78% of edema formation, which was more significant than the inhibition of Diclofenac at 0.5 mg/kg and Dexamethasone at 0.5 mg/kg (Smara, 2020).

2.4.2.3. Antimirobial activity

The REO could be used as a natural antimicrobial instead of synthetic preservatives, and due to its bioactive compounds it may be used as a natural alternative for antibiotics (Maria-Simona et al., 2017). The antibacterial activity of rosemary has been determined in various assay types based on either Minimum Inhibitory Concentration (MIC) or Minimum Bactericidal Concentration (MBC) (Neito et al., 2018). Rosemary presents different antimicrobial properties, such as antibacterial, antiviral and antifungal activities (Shiravi et al., 2021).

- **Antibacterial and antiviral activities**

In particular, rosemary extracts have been reported to exhibit strong antibacterial properties due to their chemical composition (Erkan et al., 2020). REO possessed similar antibacterial activities to α-pinene, and a little bit better than β-pinene, while 1,8-cineole possessed lowest antibacterial activities (Wang et al., 2012).

Aballah et al. (2019) reported that rosemary extracts has strong antibacterial activity against the Xoo of GZ 0005 strain, which is the pathogen of rice bacterial blight. Other studies have shown the antibacterial activity of REO against *E. coli, Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, Aeromonas hydrophyla, and Salmonella choleraesuis.*

Although rosemary and its metabolites have shown antiviral activities on different viruses. It seems that additional studies on the use of rosemary to treat viral diseases,such as SARS-CoV-2, are to be done (Shiravi et al., 2021). Carnosol is another natural occuring polyphenols found in rosemary exhibited definite anti-HIV acitivity at an early stage of virus infection, since HIV is an enveloped RNA virus like MV. Carnosol could be responsible for rosemary antiviral activity (Fraihat et al., 2015).

- **Antifungal activity**

The REO exhibited significant antifungal properties against different pathogenic fungi. It was effective against *S. sclerotiorum, S. nivalis, A. panax, and C. destructans* (Hussein et al., 2020).

Silva et al. (2020) reportedalso the antifungal activity of REO against *Aspergillus flavus*. Regarding Erkan et al. (2020), the REO has fungus-inhibiting properties against *A. niger*, which is an important disease factor in humans, plants, and animals. Moreover, Bozin et al. (2007) reported the antifungal activity of REO on Trichophyton and Microsporum species for the first time in the literature. Different studies showedalso the antifungal activity ofREOagainst the pathogenic yeast *Candida albicans,* which causes many diseases in humans (Fu et al., 2007; Akroum, 2020).

In addition, Akroum and Rouibah (2020) showed that the components of REOs, including p-cymene, linalool, γ-*terpinene,* thymol, β-pinene, α-pinene, and eucalyptol are very active against the phytopathogenic fungus *A. Alternata.*

Chapter II

The fungus *Alternaria alternata*

Chapter II. The fungus *Alternaria alternata*

1. Introduction

There are several types of microorganisms, including fungi, bacteria and viruses that cause plant diseases. Diseases caused by these pathogens (living organisms) are called biotic diseases. Also, environmental conditions, such as winter damage or drought stress can cause disease in plants, and they are known as abiotic diseases(Bak et al., 2021).Plant diseases result in an annual estimated loss of 10-15% of the world's major crops, with direct economic losses of up to hundreds of billions of dollars (Peng et al., 2021).

Fungi are among the dominant causal agents of plant diseases (Doehlemann et al., 2017). They can cause 70-80% of total plant diseases.In recent years, fungal diseases of crops have become increasingly serious as they have severely affected crop yield and quality, and they have become an important bottleneck for the development of sustainable agricultural(Peng et al., 2021).

Among these fungi, *Alternaria* is a common genus that includes saprophytic, endophytic and pathogenic species (Zhang et al., 2023). *Alternaria* species are famous as phytopathogenic fungi that cause deadly and common diseases in crops (Carrascal-Hernández et al., 2022). Leaf blight, leaf spot, black point, stem cancer, fruit rot and mouldy cores are well-known symptoms of infection by *Alternaria* species (Zhang et al., 2023).

2. The genus *Alternaria*

Alternaria is a genus of worldwide fungi found in different habitats such as soil, the atmosphere, plants or indoor environments(Sánchez et al., 2022).The genus*Alternaria*are fungi imperfecti (Deuteromycetes) belonging to the class Dothideomycetes, the order Pleosporales and the family Pleosporaceae(Li et al., 2022).

The genus*Alternaria* occupies diverse ecological niches, such as agricultural products, animals, plants, seeds, soil as well as the atmosphere (Li et al., 2022).The genusis characterized by dark colonies, colour grey to blackish-brown or black. Conidia are smooth or rough, with or without septa, arising on conidiophores. The conidiophores can be unbranched or branched, pale brown, grey, dark brown or olive and can be either solitary or grouped in bundles. The mycelium is found on the plant on the necrotic lesion (Figure 13) (Singh et al., 2016; Stuart et al., 2009, Hu et al., 2015; Lawrence et al., 2016; Melo et al., 2009; Pinto and Patriarca, 2017; Tralamazza et al., 2018).

Figure 13. Conidia and condiophores of *Alternaria* species. **(A, N):***Alternaria daucifolii* ; **(B, L, M):** *A. arborescens,* **(C, H, I, J):** *A. alternata*; **(D, O):** *A. gaisen* ; **(E):** *A. limoniasperae* ; **(F, K):** *A. tenuissima* ; **(G, P):** *A. longipes*.Scale bars = 10 µm (Woudenberg et al., 2013).

Alternaria includes many saprophytic and pathogenic species (Sánchez et al., 2022). Species of *Alternaria*arewell known as major plant pathogens causing pre- and post-harvest damage to agricultural products including cereal grains, fruits and vegetables (Patriarca et Pinto, 2018; Li et al., 2022).They naturally contaminate the aerial parts of plants and are easily isolated from decay matter. Some species cause plant diseases in the field, and others are able to colonize ripening crops as opportunistic saprophytes causing spoilage of crops after harvest and during storage (Patriarca et Pinto, 2018).

In addition to spoiling fruits and vegetables, several species of*Alternaria*produce phytotoxins that play an important role in the pathogenesis of plants(Barkai-Golan et Follett, 2017; Meena etSamal, 2019).These toxins have various negative impacts on cell organelles including chloroplast, mitochondria, plasma membrane, nucleus, Golgi bodies, etc. (Meena etSamal, 2019).

The species*Alternariaalternata* has been regarded as the major toxin-producing species. It is considered one of the most common pathogens found in a variety of natural food products including fruits and vegetables, cereal plants, seeds, and other plant organs(Barkai-Golan et Follett, 2017).

3. *Alternaria alternata*

Alternaria alternata (Fr.) Keissl (*A. alternata*), the type species for the genus *Alternaria*(Yang et al., 2022), is a common species of fungus occurring on plants as a pathogen and endophyte, and in soil as a saprophyte (DeMers, 2022).*A. alternata*, the more common species of genus*Alternaria*, is a fungus whose spores occur worldwide in two main environments. Throughout the year, the spores can be found in the organic constituents of soil. From spring to autumn, they become airborne and are therefore even more ubiquitous (Kustrzeba-Wójcicka et al., 2014). Additionally,*A. alternata* is a ubiquitous phytopathogen capable of causing diseases to >100 agricultural crops and ornamental plants (Yang et al., 2022).

3.1. A brief history of *Alternaria alternata*

The first species in the genus *Alternaria*, *A. tenuis* was described from dead plant material by Nees von Esenbeck (1776–1858) in 1816 (Figure 14), without sufficient characters for delimitation of phenotypically similar species or a surviving type specimen(DeMers, 2022). In 1912, Austrain mycologist and lichenologist Karl von Keibler (1872-1965), who continued the study of *A. tenuis*, renamed it *A. alternata*. In recognition of his research, the designation Keissl was added to the name of the fungus (Kustrzeba-Wójcicka et al., 2014).

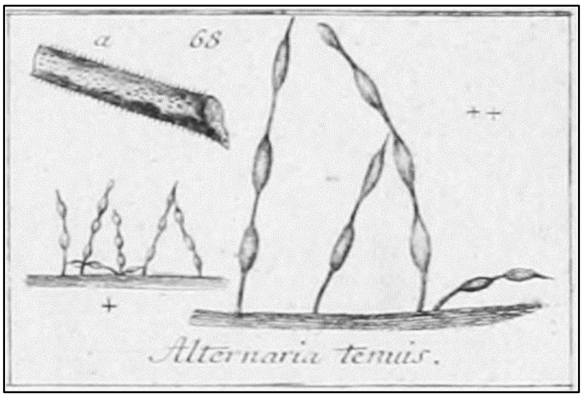

Figure 14. Nees von Esenbeck's drawing of*A. tenuis*(Kustrzeba-Wójcicka et al., 2014).

3.2. Habitat

Alternaria has a worldwide distribution, with many species being common saprophytes in soil,air and a variety of other habitats; some are ubiquitous agents of decay and plant pathogens. *Alternaria* can also be found on normal human and animal skin and conjunctiva (Pastor et Guarro, 2008).

A. alternata is a cosmopolitan species with a wide host range (DeMers et al., 2022). *A. alternata* is a prominent outdoor fungus found ubiquitously in various environments all over the globe. The saprotroph species are mostly found in the soil, plants, and foodstuffs and parasitic species are present in animals, humans, and plants.The fungal spores are airborne and usually disperse when the weather becomes dry, warm, and windy. They usually peak during summer and early autumn. *A. alternata* is also found in indoor environments, particularly in damp, water-damaged buildings, and is one of the molds associated with the “sick building syndrome”. Moreover, they can be found in textiles (carpets or beddings) and bed dust samples(Turacoz Healthcare Solutions, 2021).

3.3. Taxonomy

The taxonomy of the genus *Alternaria* has been predominately based on conidial characters, which includes shape, color, septation, and patterns of secondary sporulation, and to lesser extent on host association, biochemistry, and metabolites (Lawrence et al., 2016). There are more than 270 species in the genus *Alternaria* (Turacoz Healthcare Solutions, 2021). *A. alternata*, the most common species of this genus,represents a diverse group of saprotroph, human allergens, and plant pathogens (Armitage et al., 2020).The table (3) presented the taxonomy of *A. alternanta*.

Table 3. Taxonomy of *Alternaria alternata*(Belhamel et al., 2021).

Domain	**Eukaryota**
Kingdom	Fungi
Phylum	Ascomycota
Class	Dothideomycetes(Euascomycetes)
Order	Pleosporales
Family	Pleosporaceae
Genus	*Alternaria*
Species	*Alternaria alternate*

3.4. Morphology

A. alternata forms colonies of olive green to black colors, with a regular prominent white margin (Figure 15) (Zhang et al., 2023).The surfaces of mature colonies appear fuzzy, downy to wooly or suede like due to the presence ofnumerous hyphae.Microscopic observation shows the brown septate hyphae form conidiophores, which are also septate.Conidia are medium-brown with a short, cylindrical beak, and form long and profusely branched chains (ten or more conidia) (Figure 15) (Pastor et Guarro, 2008).The mean conidial length varied from 21.5 to 31.4 µm. However, the average conidial width varied from 10.1 to 14.3 µm for *A. alternata* (Saleem et El-Shahir, 2022).

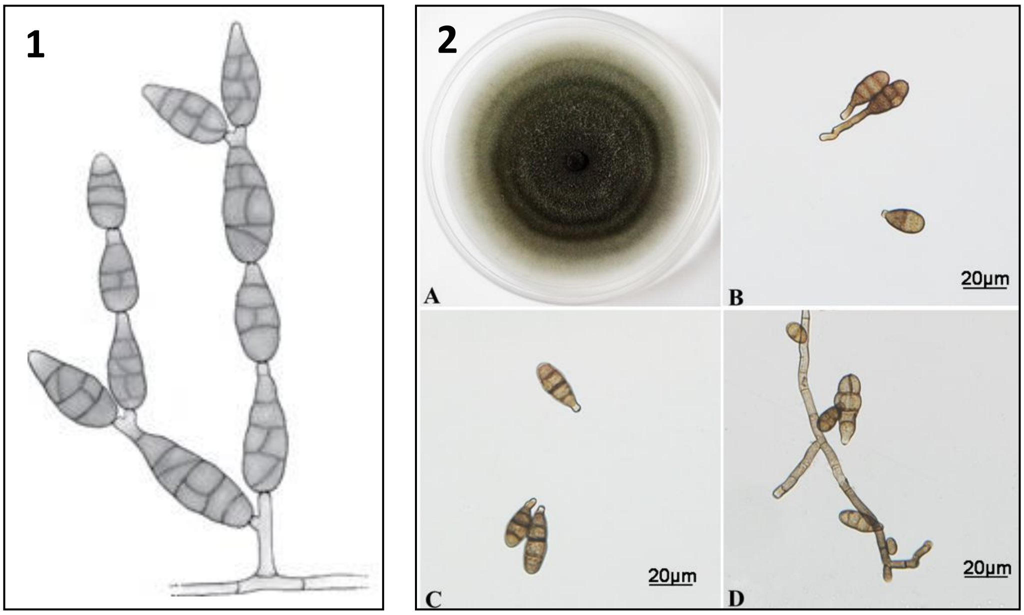

Figure 15. (1) Morphology of Conidia of *A. alternata* (Pastor et Guarro, 2008).**(2)** Morphological characterizations of *A. alternata* culturedon PCA medium: (A) Colony morphologygrown at 25°C for7 days ; (B–D) Conidia andvegetativehyphaemorphology (Zheng et al., 2017).

3.5. Growth conditions

3.5.1. Effect of temperature

Temperature is the most important physical environmental factor for regulating the growth and reproduction of fungi (Manjunath Hubballi et al., 2010). Ultra low or high temperature adversely affects the germination and growth.(Choudhary et al., 2017).

*A. alternata*could grow at different temperature levels (15, 20, 25, 30, and 35°C). Maximum mycelial growth (diameter of 88 mm) was observed at 25°C. However, the temperature of 20, 30, and 35°C resulted in 75, 71, and 65 mm mycelial growth of this fungus,respectively, but differ significantly from the growth at 25°C (Choudhary et al., 2017).

3.5.2. Effect of relative humidity

In vitro studies on different levels of relative humidity revealed that 100% relative humidity supported maximum mycelial growth of *A. alternata*, while minimum mycelial growth was observed at 50% relative humidity (Figure 16). The results are in close conformity with the observations of Prasad and Ahir (2013) who observed maximum growth of *A. alternata* at 90-100% relative humidity. In addition, Balai and Ahir (2013) also reported that 90 to 100% relative humidity was most suitable for mycelial growth of *A. alternata* (Choudhary et al., 2017).

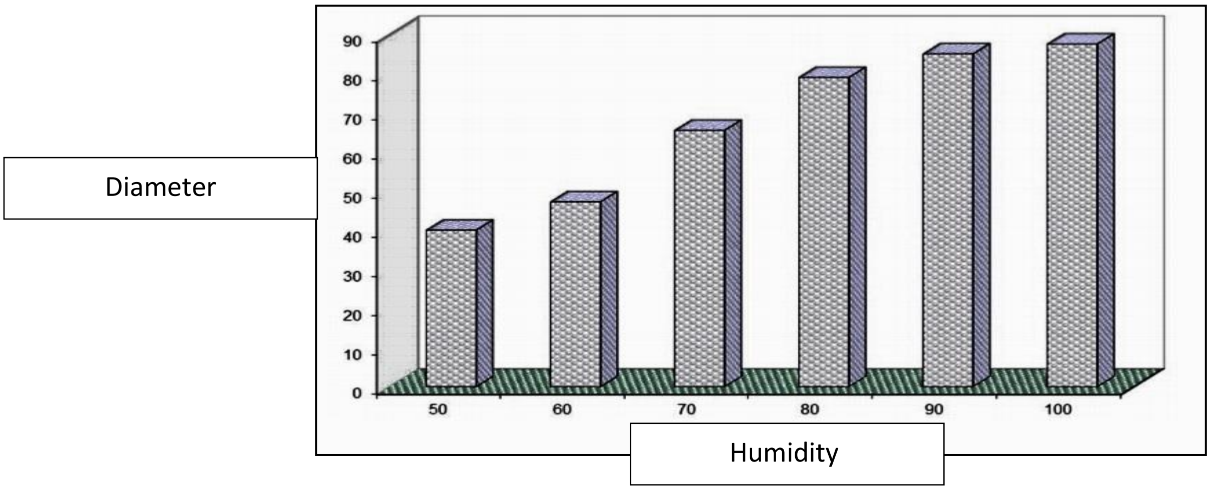

Figure 16. Effect of relative humidity on the mycelial growth of *A.alternata* at 10 days of incubation at 25°C *in vitro* (Choudhary et al., 2017).

3.5.3. Effect of pH

In general, fungi are capable of growing within a wide range of hydrogen ion concentrations of the medium while, most of them grow best in neutral or slightly acidic medium(Choudhary et al., 2017).

Fungi generally utilize substrates in the form of solution only if the reaction of solution conducive to fungal growth and metabolism. This brings importance of hydrogen ion concentration for better fungal growth. Of all the eleven pH levels, *A. alternata* prefers pH range of 6.00- 6.50. This showed that the fungus prefers acidic pH for the growth. The pH below 6.00 and above 7.00 was noticed to be inhibitory to the growth. Cochrane, Bilgrami and Verma 1978opined that in contrast to bacteria and actinomycetes, fungi are relatively tolerant to acidic ion than basic ions (Hubballiet al., 2010).

3.5.4. Effect of Culture Media

Every living being requires food for its growth and reproduction. Fungi are not an exception to it. Fungi secure food and energy from the substrate upon which they live in the nature. In order to culture the fungi in the laboratory, it is necessary to furnish those essential elements and compounds in the media, which are required for their growth and other life process. Neither all media are equally good for all fungi nor there can a universal substrates or artificial medium on nor there can a universal substrates or artificial medium on which all fungi grow well (Hubballiet al., 2010).

Physiological studies for this pathogen were carried out extensively covering media, temperature, pH and relative humidity. Out of the five media tested, potato dextrose agar proved to be the best in terms of*A. alternata*growth (Table 4). Similar results were obtained by Joshi et al. (2012), and Waghunde and Patil (2010), which reported that *A. alternata* fungus grew well on potato dextrose agar medium (Choudhary et al., 2017).

Table 4. Effect of solid media on mycelial growth of *A. alternata* at 25°C (Choudhary S et al., 2017).

Medium	Mycelial growth (mm)* after 7 days
Czapeck's medium	55.55
Martin's medium	37.77
Oat meal medium	63.33
Potato dextrose agar medium	85.55
Richard's medium	76.70

(*): Average of three replication

3.6. Phytopathogenicity of *A. alternata*

The increasing incidence of fungal infections is a rising health and economic problem for plants, people, and animals. *Alternaria* is a ubiquitous fungal genus that includes saprophytic, endophytic and pathogenic species. Some *Alternaria* species are famous as pathogens of plants and animals. In addition, those pathogenic species harm more than 4000 host plants distributed worldwide, with a broad host range, including agronomic plants, ornamentals, vegetables, fruit trees and animals. Leaf blight, leaf spot, black point, stem cancer, fruit rot and mouldy cores are well-known symptoms of infection by *Alternaria* species (Zhanget al., 2023).

A. alternata the type species for the genus *Alternaria*, is able to cause diseases in over 100 plants, including vegetables, fruits, herbs and ornamental trees (Zhanget al., 2023). This opportunistic pathogenic fungus is known to cause leaf spots, fruit rot, and stem canker in crops, such as apples, tomatoes, and pepper (Sakiyo et al., 2023). It also causes brown spot disease in all citrus producing countries (Aiello et al., 2020). In addition, a serious infection risk was posed to horticultural crops all over the world because of the rapid market globalization of the seeds, long-distance airborne transmission of spores and the influences of changed climate (Zhanget al., 2023).

3.6.1. Early blight disease

Early blight (EB) is a destructive disease affecting potato (*S. tuberosum* L.) and tomato (*Solanum Iycopersicum* L.) plants, caused by a heterogeneous group of plant pathogenic *Alternaria* fungi including *A. solani*, *A. tomatophila*, and *A. alternata*(Adhikari et al., 2017; Kokaeva et al., 2022). EB disease, caused by the ascomycete fungus *A. alternata*, is a pre- and post-harvest disease in tomato plants(Alizadeh-Moghaddam et al., 2020). It is one of the most important tomato diseases, which causes significant losses and reduces the nutritional value of tomato crop (Martinko et al., 2022).In addition, EB disease of potato is one of the major diseases responsible for massive loss in crop production. *A. solani* is considered as the main pathogen for the disease. However, *A. alternata* was also found to produce similar disease symptoms in the potato plant (Gorai et al., 2021).

Infection by this polycyclic disease led to production losses of 35 to 80% in tomato and 5 to 40% in potato (Jindo et al., 2021; Martinko et al., 2022).

- **Climate factors**

The optimum temperature for EB epidemics is in the range of 20 to 30°C. As few as 3 hours of continuous leaf wetness between 21 and 25°C is sufficient for EB lesion formation and 24± 2°C, the infection appears within 4 to 6 h of leaf wetness. There is variability with different geographic regions, with an optimal temperature in temperate regions of 22 to 28°C, while the most conidia production under tropical conditions occurs at 29 to 35°C. Radiation affects the germination of the spore (Jindo et al., 2021).

- **Diseasecycle**

The disease cycle of EB is showed in figure (17). The life cycle begins when the air temperature warms and humidity rises. Some sources marry this time frame to fruit set; however, it can vary depending on your location (Joe C, 2013).

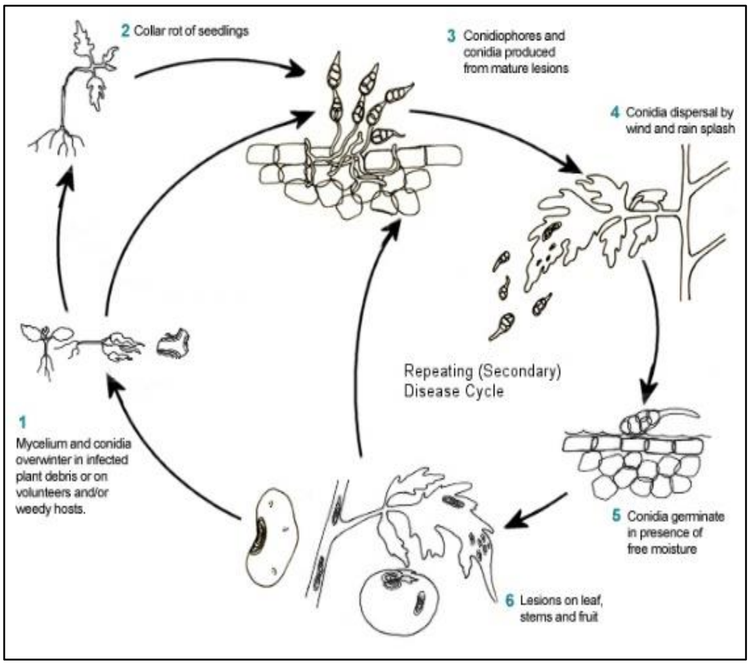

Figure 17. Disease cycle and Epidemiology of early blight (kemmit,2013).

- **Symptoms**

Early blight produces a wide range of symptoms at all stages of plant growth. These Symptoms are initially observed on older, senescing leaves. Likewise, the most susceptible plants are those that are physiologically old, weak, and malnourished, wounded by wind, sand, hail or insects (Irena et al., 2014).

The first symptoms usually appear on older leaves and consist of small, irregular, dark brown to black, dead. Early mainly affects potato foliage and leads to leaf necrosis and premature defoliation. The primary damage of early blight is due to premature defoliation of the plant. Photosynthesis rates increase and respiration rates decrease in apparently healthy tissues. Physiological changes are difficult to measure and evaluation of crop loss is based on the level of disease. Early literature cites yield losses of 5 to 50%. There is often a discrepancy between damage to foliage and yield loss, which is due to the increase in disease spread at the end of the season, when most of the yield has been produced. When tomato fruit or potato (Figure 18), tubers become infected, quantity and quality of marketable produce is decreased and the number of secondary pathogens increases. Control of early blight has been shown to increase yield (Irena et al., 2014).

Figure 18. Early blight (EB) symptoms of potato and tomato. **(A):** Apple leaflets soil showing dark brown necrotic lesion. **(B):** Tomato leaflet showing necrotic lesions dark brown, surrounded by yellowish halo. **(C):** Detail of a necrotic lesion on potato leaflet showing concentric rings reminiscent of the appearance of a target (“target spot”). **(D):** Attack on stems of tomato showing dark, lenticular leaf-like lesions. **(E):** Fruit rot tomato whose surface has a black velvety layer made up of mycelium and conidia (Ayad., 2019).

3.6.2. *Alternaria* Brown spot

Alternaria brown spot disease (ABS), which is a severe fungal disease resulting in defoliation and fruit drop of citrus, is caused by the fungus *A. alternata* tangerine pathotype. This fungus is strictly pathogenic as a result of itscapability to produce the Host-selective toxins (HST), and Adenylate cyclase toxin (ACT) (Huang et al., 2022). ABS is one of the most important diseases affecting tangerine plants and their hybrids (Perina et al., 2014). The ABS disease was first reportedon "Emperor Mandarin"in Australia in 1903, and was subsequently detected in citrus-growing regions all over the world (Cuenca et al., 2016), such as United states, South Africa, Spain, Italy and Argentina (Stuart et al., 2009).

Alternaria species cause brown spot diseases and black rot in citrus fruits are mainly due to *A. alternata* f .sp. *citri A. citri and A. alternata*. These pathogens are respossible for important damage to agricultural products both in the field and in post-harvest. They attack leaves, stems and fruits (Achetbi et al., 2021).

A. alternata has two major pathotypes: the tangerine pathotype and the rough lemon type (Chung et al., 2012). The figure (19) shows the life cycle of *A. alternata*, which causes brown spot disease.

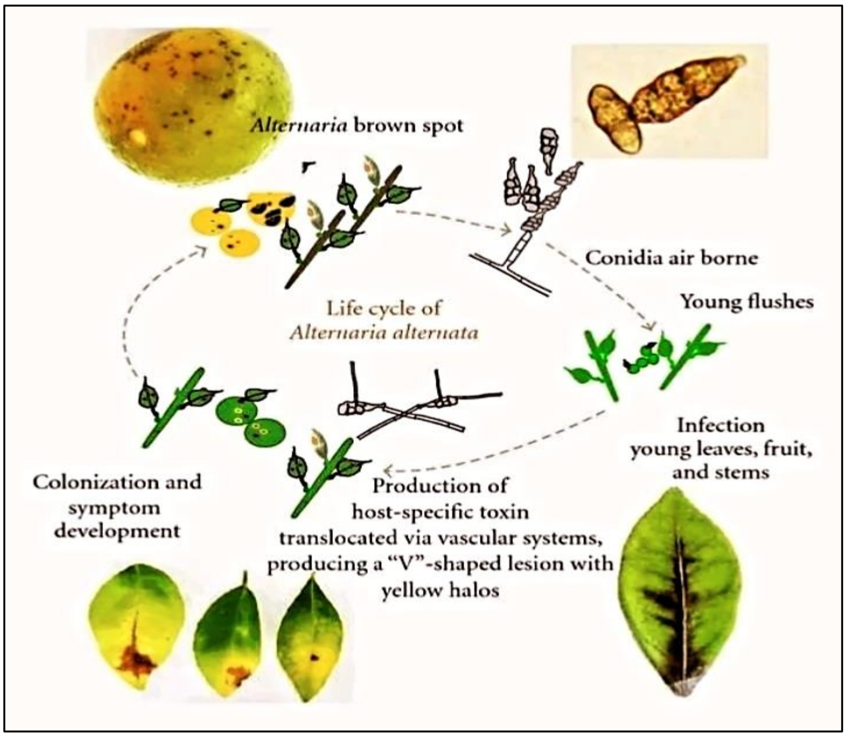

Figure 19. Life cycle of *A. alternata*, the causal agent of citrus *Alternaria*brown spot. ACT toxin produced by the tangerine pathotype of *A.alternata* is transported via the vascular system and formation of necrotic lesions on a datached calamondin leaf (bottom right) (Chung et al., 2012).

- **Symptoms of *Alternaria* brown spot**

Brown spot affects leaves, twigs and fruits. Round to oval brown spots are charactereristic symptoms of this disease (Schultz et al., 2013). Symptoms referable to *Alternaria* brown spot were observed in fields on leaves and fruits. Severe infections on mature fruit included lesions that varied from small specks to large pockmarks on the fruit surface (Figures 20a and 20b) (Vitale et al., 2021).

Figure 20. *Alternaria* brown spot. **(a):** Brown spot on "Mandalate mandarin". **(b):** Brown spot on ¨Tarocco Scire VCR). **(c):** Depressed brown to black lesions surrounded by yellow halo areas and gummosis on immature ¨Femminello Siracusano 2KR¨ lemon fruit. **(d):** Brown spot lesions with yellow halo areas on ¨Tarocco Gallo VCR¨ immature fruit. **(e):** Detail of fruit spots (specks to craters symptoms) on ¨Tarocco Sciara¨ (up) and ¨Nova mandarin" (down). **(f):** Chlorotic leaf spots on young leaf of ¨Tarocco Emanuele¨. **(g,h):**Necrotic spots with yellow halo of expanded leaves of Tarocco blood oranges (Vitale et al., 2021).

Sometimes, fruit formed a barrier of corky tissue erupting from the surface that in the later stage of the infection, can fall out forming craters (Figure 20e). On immature fruit, the symptoms included slightly depressed brown to black lesions surrounded by yellow halo areas (Figures 20c and 20d). In addition, gummosis on ¨Femminello Siracusano 2KR¨ lemon fruit was observed (Figure 20c). *Alternaria* brown spot on leaves included small, chlorotic spots on young leaves without a yellow halo (Figure 20f) that with age turned to brown or dark brown, became necrotic, enlarged, and developed yellow halos (Figures 20g and 20h). *Alternaria* brown spot on fruit determined heavy production losses with optimal environmental conditions (extended periods of leaf wetness and temperatures of less than 17°C) (Vitale et al., 2021).

3.6.3. Management of diseases

Due to the lack of cultivars resistant to this pathogen, diseases such as *Alternaria*brown spot of citrus, brown spot of tobacco, and early blight of tomato and potato are very common and cause serious economic losses. So far, treatments of these plant diseases have relied almost exclusively on application of fungicides, over the last 20 years (Yang et al., 2022). However, fungicides cannot be seen as a long-term solution because its use in agriculture caused the development of drug-resistant fungal strains. The presence of toxic residues in agricultural products may have potentially adverse effect on human health, the environment, and biodiversity (Garcia-Coronado et al., 2015)

The worldwide trend towards safe environment methods for controlling plant disease in sustainable agriculture practice needs reduced usage of synthetic chemicals. Due to this, there is an increasing interest to acquire alternative antimicrobial agents (biocontrol agents) for controlling plant diseases (Milan, 2020). For this purpose, natural fungicides of plant origin are being explored (Garcia-Coronado et al., 2015).

Several researches have recently exploited higher plant products as novel chemotherapy in plant defence, which are important sources of new agrochemicals for plant disease control. The natural products are considered to be the best as alternative to synthetic chemicals due to less negative environmental impact (Milan, 2020).

In addition, postharvest control of *A. alternata* diseases in fruits and vegetables by using conventional methods as well as a number of novel strategies including biological control, heat treatment, natural compounds (chitosan, isothiocyanates, essential oils, elicitors of natural defense mechanism) (Troncoso-Rojas et al., 2014). Essential oils and Natural plant products have been found effective in plant disease managements and could be safely incorporated as suitable alternatives for synthetic fungicides (Zaker, 2016; Hong et al., 2018). They are considered environmentally friendly preservation methods for better postharvest quality (Guo et al., 2023).

Chapter III

Report of evaluation of the antifungalactivity of thyme and rosemary essential oils

Chapter III. Report of evaluation of the antifungal activity of thyme and rosemary EOs

1. Material and Methods

The current chapter describes two research investigations based on the evaluation of the antifungal activity of the aromatic plants: thymus (*T. vulgaris* L.), androsemary (*R. officinalis* L.).

The first reseach investigation was conducted by by Grati et al. (2022), whereas the second one was performedby Shaban (2014). These experiments evaluated the effectiveness of both REOs and TEOs against the phytopathogenic fungus *A. alternata*.

The different steps of each study of them are demonstrated below.

1.1. Plant material

The plant chosen for this study is thyme (*T. vulgaris*) and rosemary (*R. officinalis* L.) (Figure 21). The part taken into consideration to carry out the present study is the aerial part (the leaves).

Figure 21. *Thymus vulgaris*leaves**(a)**(Djrourou et al., 2018) ; *Rosmarinusofficinalis*leaves**(b)** (Durant, 2021).

1.2. Plant sampling

1.2.1. Sample processing of thyme

The harvesting of the aerial part of thyme is generallyperformed in the period of May. After harvesting, the adriatic part of the plant is dried using an absorbent and clean paper, kept at room temperature in a dry place,away from light for a period of two (02) weeks (Amrouche et djaadi., 2020)

1.2.2. Sample processing of rosemary

The samples of rosemary were harvested at the flowering stage. Only the aerial part of the plant is used. The leaves and the apical parts were dried in the shade for eight days at a temperature room (Elyemni et al., 2019).

1.3. Extraction of EOs of *T. vulgaris* and rosemary *R. officinalis* L.

The different extraction techniques of EOs have been shown in the chapter one (the third part).The extraction of the EOs of *T. vulgaris* and *R. officinalis* L. is generally carried outby two techniques: hydro distillation (By Clevenger) and steam distillation. (Mnayer et al ., 2021 ; Teshale et al ., 2022)

1.4. Evaluation of antifungal activity of EOs of *T. vulgaris*and rosemary *R. officinalis* L. against the fungus *A. alternata*

1.4.1. Fruit samples (Collection)

Experiments were conducted on decayed tomato fruits (Figure 22). Harvesting was performed at maturity stage and samples were kept at 50± 5°C. The tested fruits were dipping in 3% soduim hypochlorite solution for 30 second and rinced three (3) times with sterile distilled water and left on folds of tissue papers to remove the access of water (Shaban, 2014).

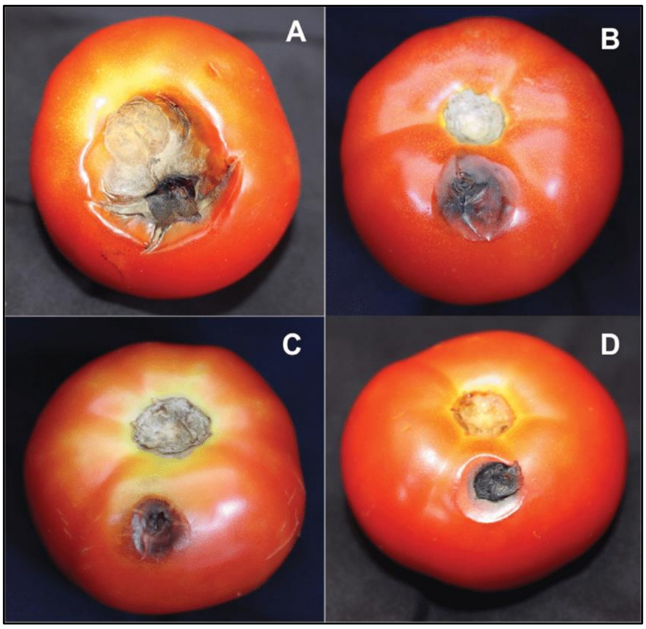

Figure 22. Infected tomato fruit **(a, b, c, d)** (Al-Maawali et al., 2021).

1.4.2. Microorganism: Fungal strain

The phypathogenic fungal strain studied in both investigations is *A. Alternata*. According to Shaban (2014), *A.alternata* was originally isolated from decayed tomato fruits (Figure 23). The pathogen was maintained on potato dextrose agar (PDA) slants(Annexe 1),and stored at 4°C. Fungal pathogenicity and virulence were maintained by inoculating tomato fruit with the fungus and re-isolating it.

With regard to Grati A et al. (2022), the strain*A. alternata* swas an isolate of 7025 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1,5,99.4% !(C. reticula-Fortune variety, BeniKhaled) Genbank accession number for nucleotide sequence: (T1) OK448177.

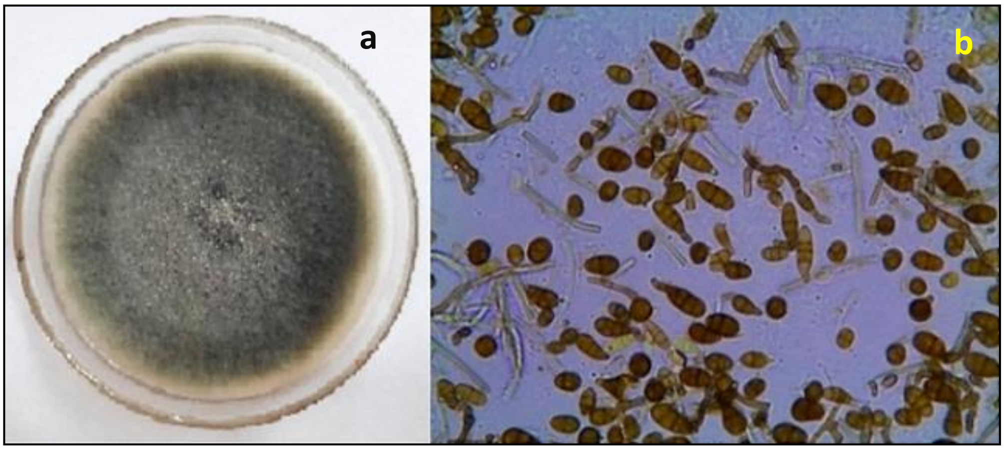

Figure 23. *Alternaria alternata*isolated from infected tomato fruits (**a**): Culture of *A. alternata* on PDA; Morphology of *A. alternata* (**b**) (Saleem and El-Shahir, 2022).

1.4.3. Effect of EOsof *T. vulgaris*and rosemary *R. officinalis* L. on *A. alternata* growth

Shaban (2014) assessed the antifungal activity of both *R. officinalis* L. and *T. vulgaris* L. against the phytopathogenic fungus *A. alternaria*. The tested EOs were used at two concentrations including 50 and 100 µl/ml.

Twenty millilitres of PDA medium containing the tested concentrations of each EO and amended with 0.5% Tween-80,were poured into Petri dishes. After solidification, the Petri plateswere inoculated withthe pathogen *A. alternaria* and incubated at 25°C for 14 days.

After incubation, the growth reduction was calculated in relative to check treatment (control) using the following equation according to Fokemma (1973):

Reductionpercentage (%) = (C-T) /C x 100

Whereas:

C= Maximum linear growth in control.

T = Maximum linear growth in treatment.

In the investigation conducted byGrati et al. (2022), the antifungal activities of leaf EOs from *R. officinalis* and *T. vulgaris* were also assessed.In the study by Grati et al. (2022) also evaluated the antifungal activity of R. officinalis and T. vulgaris leaves EO. Spore suspensions of *A. alternata* (in 0.01% Tween 80 medium) were prepared from 8-day-old single-bead cultures from several

Eppendorf tubes containing different doses of EO from 0 to 12 mg/ml (with 0.5 % Tween 20 solution) in PDB medium. Then, a volume of 20 µl of spore suspension (10^{-5})was added.

Finally, the tubes were incubated with shaking in the dark at 25°C. The Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the EO required to completely prevent visible fungal growth.

In addition, a portion of the *A. alternata* culture kept in a 20% glycerol solution at -20°C was removed and incubated on a PDA at 20°C in the dark. Sporulation was stimulated using the method of Zerigui and Mouzaoui (2018). After 10 days of incubation, place the dishes at 4°C for 1 hr. They were then exposed to direct light for 3 hours and allowed to come to room temperature in the dark for 24 hours. Finally, a spore suspension was obtained after scraping the culture of *A. alternata.*

2. Result and discussion

2.1. Effect of thyme and rosemary EOs on fungal growth inhibition

The efficienty of *R. officinalis* and *T. vulgaris* EOs on *A. alternaria* growth reduction was evaluated by Shaban (2014).

The obtained results of this study indicated that *T. vulgaris* EO showed the highest percentage of fungal growth reduction, followed by *R. officinalis* EO (Table 5). *T. vulgaris* EO exhibited most pronounced antifungal potentials against the pathogen*A.alternata*as it produces 53.4% of mycelial growth reduction at concentration of 50µl/100 ml after 7 days of incubation. Whereas at concentration of 100µl/100ml, the *T. vulgaris* EO inhibited 74.9% of mycelial growth of *A. alternata* (Table 5). *R. officinalis*EO showed less antifungal activity against the pathogen *A. alternaria*. With growth reduction percentages of 36.2 and 58.2% at concentrations of 50 and 100µg/100ml, respectively.

Consequently, both EOs exhibited a significant negative impact on mycelium growth of tested fungus compared with the control treatment.

Table 5. Effect of two concentrations of thyme and rosemary EOs on linear growth of *A. alternata* (Shaban, 2014).

Treatment	50µl/100ml		100µl/100ml	
	Linear growth (mm)	Growth reduction (%)	Linear growth (mm)	Growth reduction (%)
Thyme (*T. vulgaris*) EO	42.0	53.4	22.6	74.9
Rosemary (*R. officinalis*) EO	57.5	36.2	37.7	58.2
Control	90.0	-	90.0	-

2.2. Determination of MIC values of *T. vulgaris* and *R. officinalis* EOs

In the investigation conducted by Grati et al. (2022), the antifungal activity of leaf EOs from the aromatic plants *T. vulgaris* and *R. officinalis*were tested against the pathogen *A. alternata*. The minimal concentration of these EOs, ranged between 5000 and 8000 µg/ml, completely inhibited the growth of *A. alternata*.

The results of MIC determination (Table 6) showed that *R. officinalis* EO was the most effective against the pathogen *A. alternata*, with a MIC value of 5000 µg/ml. Moreover, the obtained results indicated that *T. vulgaris*EO exhibited also an antifungal activity against the fungal pathogen with a MIC value of 6000 µg/ml.

Table 6. Minimum inhibitory concentration (MIC) of leaf EOs from *Rosmarinus officinalis* and *Thymus vulgaris* (Grati et al., 2022).

Species	MIC (µg/ml)	
	***R. officinalis* EO**	***T. vulgaris* EO**
A. alternata	5000	6000

These findings contrast with the previous results reported by Shaban (2014) where *T. vulgaris*EO showed a higher antifungal activity against the species *A. alternata* as comprared to *R. officinalis* EO. Different studies (Bellumori et al., 2021; Akroum et al., 2020) reported that the leaf EOs of rosemary have the highest antifungal activity, followed by thyme EOs. Rosemary leaf EOs were rich in 1,8-cineole, whereas carvacrol was the main component of thyme EOs.

The composition of EOs within the same botanical species is influenced by various parameters such as time of harvest, mode of extraction, environmental conditions in which the plant grew, dry periods during the vegetative phase, water deficit, exposure to the sun, climate conditions, salt stress, and other conditions can influence the production and chemical composition of EOs. Hence, differences in yield could be result from the influence of these external factors (Mancianti and Ebani, 2020; Jazo et al., 2023).

On the other hand, the results of Shaban (2014) are in agreement with previous studies (Soliman and Badea, 2002; Edris and Ferrag, 2003, and Ćosićet al., 2010), as thyme EO is characterized by strong toxinc propertiesits active compounds,such as thymol and carvacrol, against a large number of microorganisms. Moreover, Imelouane et al. (2009) extracted two major chemical compounds from the thyme plant leaves, which can be considered as effective antifungal agents providing permanent damage to the mycelial growth of fungi.

Additionnally,Šegvićetal.(2007) reported that the thyme EO containsp-cymene (36.5%), thymol (33.0%) and 1,8-cineole (11.3%) as main components, and pure thymol exhibited antifungal activities. In the investigation conducted by Plotto*et al.* (2003), *in vitro* tests showed that maximum inhibition of fungal growth (100%) was obtained after the inoculation of *A. alternata* on agar plates containing 500 mg/l of thyme EO.

Thymol is the major active component of thyme EO, which might have a fungicidal activity that probably resulted in extensive damage to the cell wall and cell membrane of the fungi (Aslam et al ., 2022)

Furthermore, Bartyska and Budzikuer-Ramza (2001) described high toxicity of rosemary (44.40% of 1,8 cineole) against *Fusarium* spp.Akroum et al. (2020) indicated that the methanolic extract of *R. officinalis* showed the greatest antifungal activity *in vitro*as well as on cultivated plantsunder a greenhouse. On agar plates, this extract has been shown to acton *A. alternata* with a complete inhibition ofdictyospores formation. These findings were confirmed by the absence of these sporesin samples taken from fruit treated with the tested extract. The plant species*Punica granatum* and *Lavandula angustifolia*showed also a good activity on *A. alternata*, but it was lower thanthat of *R. officinalis*.

On the other hand, Farooq et al. (2022) found that thyme EO was significantly ($p \leq 0.05$) effective against major postharvest fungal pathogens of loquat *(Eriobotrya japonica)* showing a maximum growth inhibition against *A. alternata* (68.15%) at concentration of 0.06%.

Large percentages of antifungal activities of rosemary oil are related with α-pinene of monoterpens as the main compound (Moghtader et al., 2011). Akroum and Rouibah (2020) showed that the components of REO, including p-cymene, linalool, γ-terpinene, thymol, β-pinene, α-pinene, and eucalyptol, were effective against the phytopathogenic fungus A .alternata is very effective.

The antifungal ability of EOs is due to the presence of chemical compounds, whichact as a natural fungicide against various fungal pathogens inhibiting fungal activity (Aslam et al., 2022)

Other investigations have also reported the inhibitory effect of some EOs, which have potential antifungal properties also have the possibility for use as alternatives to synthetic fungicides.

In conclusion, we can say that EOs of *T. vulgaris* and *R. officinalis* showed strong ability aspotential antifungal agents for controlling plant disease caused by the pathogen *A. alternata*.

Conclusion

Conclusion

In conclusion, the research on the antifungal activity of *Thymus vulgaris L.* (thyme) and *Rosmarinus officinalis L.* (rosemary) against *Alternaria alternata* demonstrates their significant potential as natural fungicides. The bioactive compounds found in these herbs, such as thymol, carvacrol, rosmarinic acid, camphor, and cineole, exhibit strong inhibitory effects on the growth and development of *Alternaria alternata.*

Thyme and rosemary extracts disrupt the fungal cell membrane, interfere with crucial metabolic processes, and induce oxidative stress, leading to the inhibition of fungal growth. These findings suggest that thyme and rosemary could serve as effective alternatives to synthetic fungicides for managing *Alternaria alternata* infections in agricultural crops.

The eco-friendly nature of thyme and rosemary extracts further adds to their appeal, as they offer a sustainable and environmentally conscious approach to crop protection. By utilizing these natural compounds, farmers can reduce the reliance on synthetic chemicals and minimize potential harm to the environment.

However, further studies are needed to optimize extraction methods, determine the most potent active compounds, and develop appropriate formulations for practical application in the field. Additionally, research exploring the potential synergistic effects of thyme and rosemary with other natural compounds or conventional fungicides could enhance their efficacy against *Alternaria alternata* and other pathogenic fungi.

In summary, the antifungal activity of *Thymus vulgaris L.* and *Rosmarinus officinalis L.* against *Alternaria alternata* provides a promising avenue for sustainable agriculture. Continued investigation and development of these natural compounds will contribute to the advancement of environmentally friendly strategies for managing fungal diseases in crops, promoting healthier and more productive agricultural systems.

Bibliographic reference

References

Abdelli, W., 2017. Chemical characterization and study of some biological activities essential oils of Juniperus phoenicea and Thymus vulgaris. Doctoral thesis: Applied Microbiology. Abdelhamid IbnBadis University.Mostaganem.

Aboura, M., Belguendouz, A., 2021. Etude de l’activité antifongique des huiles essentielles de deux plantes aromatiques Rosmarinussp.etMyrtussp. vis-à-vis du champignon Fusariumsp(Master Thesis). Université Abdelhamid Ibn-Badis. Mostaganem.

Achetbi H., Amiri S., Lahlali R., 2021. Les Alternaroises *(Alternaria spp.)* des argumes : Diagnostic et méthodes de lutte. *Marocaine des Sciences Agronomiques et Vétérinaires,* 9 (2), 158-169.

Adhikari, P., Oh, Y., Panthee, D.R., 2017. Current Status of Early Blight Resistance in Tomato. Internationial journal Molecular Plant Sciences 18(10), 1-22.DOI: org/10.3390/ijms18102019.

Aiello, D., Guarnaccia, V., Azzaro, A., Pollizi, G., 2020. Alternaria brown spot on new clones of sweet orange and lemon in Italy. PhytopathologiaMediterranea 59(1), 131-145.

Akroum, S., Rouibah, M., 2020. Utilisation d’extraits méthanoliques de plantes pour la protection des cultures de tomates-cerises (Solanumlycopersicum var. cerasiforme) contre l’infection fongique par Alternariaalternata. Biologie Aujourd’hui 214(1-2), 55-61. DOI : 10.1051/jbio/2020001.

Akthar,M.S., Degaga, B., Azam, T., 2014.Antimicrobial activity of essential oils extracted frommedicinal plants against the pathogenic microorganisms.Issues in Biological Sciences and Pharmaceutical Research2 (1), 1-7

Alizadeh-Moghaddam, G., Rezayatmand, Z., Esfahani, M.N., Khozaei, M., 2020. Bio-genetic analysis of resistance in tomato to early blight diseases, *Alternaria alternata.*Breeding science 72(4), 286-296.DOI: 10.1270/jsbbs.22003.

Almeida-Couto J. M. F., Ressutte J. B., Cardozo-Fihlo L., Cabral V. F., 2022. Current extraction methods and potential use of essential oils for quality and safety assurance of food. An Acad Bras Cienc, 94 (2), 1-20. DOI 10.1590/0001-3765202220191270

Alu’datt, M.H., Rababah, T., Alhamad, M.N., Gammoh, S., Al-Mahasneh, M.A., Tranchant,C.C., Rawshdeh, W., 2018. Pharmaceutical, Nutraceutical andTherapeutic Properties of Selected WildMedicinal Plants: Thyme, Spearmint, andRosemary. Therapeutic Foods and Ingredients Vol ,275-290.DOI:org/10.1016/B978-0-12-814625-5.00014-5.

Andrade J. M., Faustino C., Garcia C., Ladeiras D., Reis C. P., Rijo P., 2018. *Rosmarinus officinalis L.* : an update review of its phytochemistry and biological activity. *Future Science OA*, 4 (4), 1-18.

Armitage, A.D., Cockerton, H.M., Sreenivasaprasad, S., Woodhall, J., Lane, CH.R., Harrison, J.R., Clarkson, J.P., (2020). Genomics Evolutionary History andDiagnostics of the *Alternariaalternata* Species Group IncludingApple and Asian Pear Pathotypes. Frontiers in Microbiology 10(3124), 1-17.DOI: 10.3389/fmicb.2019.03124.

Aslam, M.F., Irshad, G., Naz, F., Khan, A.M., 2022.Evaluation of the antifungal activity of essential oils against *Alternaria alternata* causing fruit rot of *Eriobotrya japonica*.TurkishBiochemical society 47(4), 511-521 .

Ayad, D., 2019. Research on the causative agents of potato *alternaria* and tomato in Algeria. Comparative analysis of their diversity phenotypic and genotypic according to cropping systems and floors bioclimatic. Doctoral thesis: Biotechnology applied to Phytopathology. ENSA d'El Harrach. EL Harrach –Alger.

Aziz Z. A. A., Ahmad A., Setapar S. H. M., Karakucuk A., Azim M. M., Lokhat D., Rafatullah M., Ganash M., Kamal M. A., Ashraf G. M., 2018. Essential oils : Extraction techniques, Pharmaceutical and therapeutic potential- A review. *Current Drug Metabolism,* 19 (00), 1-11.

Baptista-Silva, S., Borges, S., Ramos, L.O., Pintado, M., Sarmento, B., 2020., The progress of essential oils as potential therapeutic agents.Centro de Biotecnologia e Quimica Fina (CBQF) Vol, 1-35.

Borges R. S., Ortiz B. L. S., Pereira A. C. M., Keita H., Carvalho J. C. T., 2019. *Rosmarinus officinalis* essential oil : Areview of phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J*ournal of Ethnopharmacology,* 229 (n°), 29-45. https://doi.org/10.1016/j.jep.2018.09.038

Carović-Stanko K., Petek M., Grdiša M., Pintar J., Bedeković D., ćustić M. H., Satovic Z., 2016. Medicinal plants of the family Lamiaceae as functional foods-A review. *Food Sci,* 34 (5), 377-390. Doi : 10.17221/504/2015-CJFS

Chelbab, B., Mihoub, N., 2019., Evaluation de l'activité de l'huile essentiellede *Thymus vulgaris* vis-à-vis*d'Alternariaalternata*, moisissure de blé de stockagenon traité. Master'sthesis :Microbiologie appliquée. Université Mohamed El Bachir El Ibrahimi- B.B.A. 30p

Choudhary, S., Ghasolia, R., Yadav, A., Kakraliya, L., Nasanwa, R., 2017. Effect of Physical Factors on Growth of Alternaria Alternata Inciting Leaf Blight of Isabgol. Chem Sci Rev Lett 6 (22), 857-863. DIO: CS202048031.

Chung, K.R., 2012. Stress Response and Pathogenicity of the Necrotrophic Fungal Pathogen *Alternaria alternata.* Institute of Food and Agricultural Sciences (IFAS) Vol 2012,1-17

Concalves, C., Fernandes, D., Silva, I., Mateus, V., 2022. Potential Anti-Inflammatory Effect of Rosmarinus officinalis in Preclinical in Vivo Models of Inflammation. Molecules 27(3): 609. DOI: 10.3390/molecules27030609.

Dauqan E. M., Abdullah A., 2017. Medicinal and functional values of thyme *(Thymus vulgaris L.)* herb. *Journal of Applied Biology & Biotechnology,* 5 (02), 017-022.

DeMers, M., 2022. *Alternaria alternata* as endophyte and pathogen. Microbiology. 2022 (168). 1-15.DOI: 10.1099/mic.0.001153.

Denita, S., Kumar, A., 2020. Recent Methods to Control Early Blight of Tomato *(Solanum lycopersicum L.)*. Plant Cell Biotechnology and Molecular Biology 21(37&38), 136-148

Devika M., 2021. On overview of medicinal and aromatic plants. *Medicinal & Aromatic Plants,* 10 (386), 1.

Dikme, T., 2023. Use of medicinal and aromatic plants in food. Eu Clin Anal Med11(1), 6-10. DIO : 10.4328/ECAM.10039.

Erkan I. E., Asci O. A., 2020. Studies on Antimicrobial, Antifungal, Antioxidant properties of rosemary : A review. *Turkish Journal of Agriculture-Food Science and Technology,* 8 (12), 2708-2715.

Fani M., Kohanteb J., 2017. In vitro antimicrobial activity of *Thymus vulgaris* essential oil against major oral pathogens. *Journal of Evidence-Based Complementary & Alternative Medicine,* 22 (4), 660-666. https://doi.org/10.1177/2156587217700772

Fraihat A., Khalil M., Bustanji Y., 2015. Bioactivity of rosemary and sage against Measles. *International Journal of Science : Basic and Applied Research,* 24 (1), 222-233.

Garcia-Coronado, H., Troncoso-Rojas, R., Tiznado-Hernàndez, M.E., Carmzn de la Cruz Otero, M., Diaz-Camacho,S.P., Bàez-Flores, M.E., 2015. Analysis of a suppressive subtractive hybridization library of*Alternaria alternata* resistant to 2-propenyl isothiocyanate.Electronic Journal of Biotechnology 18 (2015), 320-326

Gonçalves, C., Fernandes, D., Silva, I., Mateus, V., 2022. Potential Anti-Inflammatory Effect of Rosmarinus officinalis in Preclinical In Vivo Models of Inflammation. Molecules 27(609), 2-16

González-Minero, F., Bravo-Díaz, L., Ayala-Gómez, A., 2020. Rosmarinus officinalis L. (Rosemary): An Ancient Plant with Uses in Personal Healthcare and Cosmetics. Cosmetics 7(4), 77.

Gonzalez-Miniro, F., Bravo-Diaz, L., Ayala-Gomze, A., 2020.*Rosmarinus officinalis* L. (Rosemary): An Ancient Plant with Uses in Personal Healthcare and Cosmetics. Cosmetic 7(4). 77. DOI: 10.3390/cosmetics7040077.

Gorai, S.P., Ghosh, R., Konra, S ., Mandal, Ch.M., 2021.Biological control of early blight disease of potato caused by *Alternaria alternata* EBP3 by an endophytic bacterial strain *Bacillus velezensis* SEB1.Biological Control Vol 156. DOI:org/10.1016/j.biocontrol.2021.104551.

Grati, A., Lasramb, S., Hammamia, M., Yeddesa, W., Wannesa, W., 2022. A comparative assessment of antifungal activity of essential oils of five medicinal plants from Tunisia. PLANT BASED PHARMACEUTICALS. PLANT BASED PHARMACEUTICALS 2(2), 2020-227. DOI: https://doi.org/10.29228/ijpbp.4.

Habib N., Regagba Z., Miara M.J., Ait Hammou M., Snorek J., 2020. Floristic diversity of steppe vegetation in the region of Djelfa, Nourth-West Algeria. *Acta Botanica Malacitana*, 45 (n°), 37-46. DOI: http:// dx.doi.org/10.24310/abm.v45i0.7987 Jindo ,K., Evenhuis ,A., Kempenaar, C., Sudré ,C., Zhan, X., Teklu, M., Kessel, G., 2021.Holistic pest management against early blight disease towards sustainable agriculture. Society of Chemical Industry Vol77, 3871-3880

Hammodi Halat D., Krayem M., Khaled S., Younes S., 2022. A focused insight into thyme : Biological, chemical, and therapeutic properties of an Indigenous Mediterranean Herb. *Nutrients*, 14 (2104), 1-22.

Hammoudi Halat, D., Krayem, S., Khaled, S., Samar, Y., 2022 .A Focused Insight into Thyme: Biological, Chemical, and Therapeutic Properties of an Indigenous Mediterranean Herb. NutrientsVol (14), 1-22

Hong J. K., Jo Y. S., Ryoo D. H., Jung J. H., Kwon H. J., Lee Y. H., Chang S. W., Park C-J., 2018. *Alternaria* spots in tomato leaves differently delayed by four plant essential oil vapours. *Research in Plant Disease*, 24 (4), 292-301.

Hossain M. A., Alrashdi Y. B. A., Al-Touby S. S. J., 2022. A review on essential oil analyses and biological activities of the traditionally used medicinal plant *Thymus vulgaris L*. *International Journal of Secondary Metabolite*, 9 (1), 103-111. https://doi.org/10.21448/ijsm.1029080

Hossain, M.A., Abdullah Alrashdi, Y., Jaroof Al-Touby, S., 2022. Essential oil analyses and biological activities of the traditionally used medicinal plant *Thymus vulgaris L*. International Journal of Secondary Metabolite 9 (1), 103-111

Hosseinzadeh S., Jafarikukhdan A., Hosseini A., Armand R., 2015. The application of medicinal plants in traditional and modern medicine : A review of *Thymus vulgaris*. *International Journal of Clinical Medicine*, 6 (n°), 635-642.http://dx.doi.org/10.4236/ijcm.2015.69084

Huang S., Jia Z., Li H., Zhang S., Shen J., Gai Y., Jiao C., Sun X., Duan S., Wang M., Ma H., 2022. ACT-Toxin, the key effector for the virulence of *Alternaria alternata* tangerine pathotype to specific citrus species. *Agronomy*, 12 (3181), 1-11.

Hubballi, M., Nakkeeran, S., Raguchander, T., Anand, T., Samiyappan, R., 2010. Effect of Environmental Conditions on Growth of Alternaria alternata Causing Leaf Blight of Noni. World Journal of Agricultural Sciences 6(2), 171-177.

Immaroh N. Z., Kuliahsari D. E., and Nugraheni S. D., 2021. *Eucalyptus globulus* essential oil extraction method. *IOP*, vol (n°) , 1-7. doi:10.1088/1755-1315/733/1/012103

Jain, N., Choudhary, P., 2022. Phytochemistry, Traditional Uses and Pharmacological Aspect of *Thymus vulgaris*.Indian Journal of Pharmaceutical Sciences 84(6),1369-1379.

Jažo, Z., Glumac, M., Paštar,V., Bekti´c, S., Radan, M., Carev, I., 2023.Chemical Composition and Biological Activity ofSalvia officinalis L. Essential Oil. Plants12(1794), 1-16. DOI: org/10.3390/plants12091794.

Joshi, D., Patel, R., Jaiman, R., Patel, N., 2012. Physiological studies of Alternaria alternata causing leaf spot and fruit rot of papaya. Environ and Ecol.30, 37-40.

Kaur Virk Pannu S., Kaur H., Kaur K., Singh V., 2018. A comparative study of various oil extraction techniques in plants : A review. Agriways, 6(2), 59-65.

Kemmitt, G., 2013. Early blight of potato and tomato. The Plant Health Instructor. DOI: 10.1094/PHI-I-2002-0809-01.

Kokaeva, L.Y., Yarmeeva,M.M., Kokaeva, Z.G., Chudinova, E.M., Balabko, P.N., Elansky, S.N., 2022 Phylogenetic Study of *Alternaria*Potato and Tomato Pathogens in Russia. Diversity 14 (685). 1-11. DOI:org/10.3390/d14080685.

Kompelly, A., Kompelly, S., Bakshi, V., Boggula, N., 2019. *Rosmarinus officinalis L*: an update review of its phytochemistry and biological activity. Journal of Drug Delivery and Therapeutics 9(1), 323-330

Kuete, V., 2017. Medicinal species and vegetables from Africa : Therapeutic potential against metabolic, inflammatory, infections and systematic diseases. Cameron : Elsevier. 669. –Academic Press.

Kumar P., Singh S., 2022. Extraction and therapeutic potential of essential oil. International Journal of Creative Research Thoughts, 10 (2), 887-896.

Kustrzeba-Wójcicka I ., Siwak E., Terlecki G., Wolańczyk-Mędrala A., 2014. *Alternaria alternata* and its allergens : A comprehensive review. *Clinical Reviews in Allergy & Immunology,* vol (n°), 1-13. DOI 10.1007/s12016-014-8447-6

Kustrzeba-Wójcicka, I., Siwak, E., Terlecki, G., Wolańczyk-Mędrala, A., Mędrala, W., 2014. *Alternaria alternata* and Its Allergens: a Comprehensive Review. Clinic Rev Allerg ImmunolVol, 1-11.DOI : 10.1128/spectrum.00382-22.

Lawrence1 P.D., Rotondo, F., Gannibal, P.B., 2015. Biodiversity and taxonomy of the pleomorphic genus *Alternaria.*Mycol Progress15(3), 1-22.DOI: 10.1007/s11557-015-1144-x

Li, J., Phookamsak, R., Jiang, H., Jayarama Bhat, D., Camporesi, E., Lumyong, S., Kumla, J.,Hongsanan, S., Mortimer, P.E.,Xu, J., Suwannarach, N., 2022. Additions to the Inventory of the Genus *Alternaria* Section*Alternaria (Pleosporaceae*, *Pleosporales)* in Italy. Journal of Fungi8 (898), 1-22.DOI: org/10.3390/jof8090898

Lorenzo J. M., Khaneghah A. M., Gavahian M., Marszalek K., Es I., Muneketa P. E. S., Ferreira I. C. F. R., Barba F. J., 2018. Understanding the potential benefits of thyme and its derived products for food industry and consumer health : From extraction of value-added compounds to the evaluation of bioaccessibility, biovialability, anti-inflammatory, and antimicrobial activities. *Critical Reviews in Food Science and Nutrition,* vol (n°), 1-17.

Mallappa, K.S., Akhtar, M.S., Sinniah, U.R., 2016. Antimicrobial Properties of Plant Essential Oils against HumanPathogens and Their Mode of Action.Evidence-Based Complementary and Alternative MedicineVol (2016), 1-26.

Mancianti, F., Ebani, V.V., 2020.Biological Activity of Essential Oils. Molecules25 (678), 1-4 DOI:10.3390/molecules25030678.

Marion, L., 2017. Le Romarin, Rosmarinusofficinalis L., une Lamiacée médicinale de la garrigue provençale. Doctoral thises. Pharmacological faculty. University of Aix-Marseille.

Martinko, K., Ivankovi´c, S., Lazarevi´c, B., Đermi´c, E., Đermi´c, D., 2022. Control of Early Blight Fungus *(Alternaria alternata)* in Tomato byBoric and Phenylboronic Acid. Antibiotics 11(320), 1-12. DOI: org/10.3390/antibiotics11030320.

Medjaher H. E. S., Benchohra A. H., Ayache A., 2021. Ethnobotanical investigation and phytochemical screening of two species of the lamiaceae family in el-bayadh province (Western of Algeria). *Ukrainian Journal of Ecology*, 11 (8), 134-139.

Meenaa, M., Samal, S., 2019. *Alternaria* host-specific (HSTs) toxins: An overview of chemicalcharacterization, target sites, regulation and their toxic effects. Toxicology Reports 6 (2019), 745–758.DOI :org/10.1016/j.

Micic, D., Đurovic, S., Riabov, P., Tomic, A., Šovljanski, O., Filip, S., Tosti, S., cinovic, B., Božovic, R., Jovanovic, D., and Blagojevic, S., 2021. Rosemary Essential Oils as a Promising Source of Bioactive Compounds: Chemical Composition, Thermal Properties, Biological Activity, and Gastronomical Perspectives. Foods 10 (11), 2734. DOI: 10.3390/foods10112734.

Mohamed, A.A., Alotaibi M.B., 2022. Essential oils of some medicinal plants and their biological activities. UMM AL-QURA UNIVERSITY Vol 9, 40-49

MOUAS, Y., 2018. Effet comparatif des parametres physiologiques, biochimiques et therapeutiques de romarin rosmarinusofficinalis l. Doctoral thesis : agronomic sciences. Saad Dhlebuniversity of blida.

Mouffouk, K.M., 2021. Quality evaluation and biological properties of Algerian commercial honeys labeled as Rosemary, Tamarisk, Thistle and Multiflora. Master’s thesis: Biotechnological Engineering. University Moulay Taher. Algeria.

Neito G., 2020. A review on applications and uses of *Thymus* in the food industry. *Plants*, 9 (961), 1-29. Doi : 10.3390/plants9080961

OUTALEB, T., 2016. Extraits de romarin d'Algérie (Rosmarinusofficinalis L. et Rosmarinustournefortii De Noe). Doctoral thesis. National School of Agronomy - El Harrach-Algiers.

Papaachan, F., Suku, M., Mohanan, S., 2023.*Rosmarinus officinalis*: Herbs, Spices and Their Roles in Nutraceuticals and Functional Foods. Academic Press, pp. 149-170.

Pastor,F.J., Guarro,J., 2008. *Alternaria* infections: laboratory diagnosis and relevant clinical features. Clin Microbiol Infect 2008 (14), 734–746.DOI: 10.1007/s12016-014-8447-6

Patil S. M., Ramu R., Shirahatti P. S., Shivamallu C., Amachawadi R. G., 2021. A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of *Thymus vulgaris* Linn. *Journa Heliyon,* 7 (n°), 1-22.

Patil, Sh.M., Ramu, R., Shirahatti P.S., Shivamallu, C., Amachawadi R.G., 2021. A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of *Thymus vulgaris* Linn. Heliyon 7(2021), 1-22

Patriarca, A., Ferna ́ndez Pinto, V., 2018. *Alternaria.*Laboratorio de Microbiología de AlimentosVol,1-8.DOI : 10.1111/j.1469-0691.2008.02024.

Peng Y., Li S. J., Yan J., Tang Y., Cheng J.P., Gao A. J., Yao X., Ruan J. J., Xu B. L., 2021. Research progress on phytopathogenic fungi and thier role as biocontrol agents. *Frontiers in Microbiology,* 12 (670135), 1-13. Doi : 10.3389/fmicb.2021.670135

Perina F. J., Amaral D. C., Fernandes R. S., Labory C. RG., Teixeira G. A., Alves E., 2014. *Thymus vulgaris* essential oil and thymol against *Alternaria alternata* (Fr.) Keissler : effects on growth, viability, early infection and cellular mode of action. *Society of Chemical Industry,* vol (n°), 1-8.

Pieracci, Y., Ciccarelli, D., Pistelli, L.,Flamini,G., Giovanelli, S., Cervelli, C, Mancianti,F., Nardoni, S., Bertelloni, F., Virginia Ebani, V., 2021.Antimicrobial Activity and Composition of Five *Rosmarinus* (Now Salvia spp. and Varieties) Essential Oils. Antibiotics10(1090),1-12.1090. DOI: org/10.3390/.

Porte A., Godoy R. L. O., 2008. Chemical composition of *Thymus vulgaris L* (thyme) essential oil from the Rio de Janeiro State (Brazil). Journal of the Serbian Chemical Society, 73 (3), 307-310. doi: 10.2298/JSC0803307P

Posgay M., Greff B., Kapcsandi V., Lakatos E., 2022. Effect of *Thymus vulgaris L.* eesential oil and thymol on the microbiological properties and meat products : A review. *Heliyon,* 8 (2022), 1-10.

Rahbardar, M., Hosseinzadeh, H., 2020. Therapeutic effects of rosemary (Rosmarinus officinalis L.) and its active constituents on nervous system disorders. Iran J Basic Med Sci 23(9), 1100-1112. DOI: 10.22038/ijbms.2020.45269.10541.

Raveau R., Fontaine J., Sahraoui A. L-H., 2020. Essential oils as potential alternative biocontrol products against plant pathogens and weeds : A review. *Foods,* 9 (365), 1-31. DOI : 10.3390/foods9030365

Rehab, A., 2021. Sustainability of solvent extraction techniques in pollution prevention and control: Handbook of Advanced Approaches towards Pollution Prevention and Control. Elsevier, pp. 311-322.

Sakiyo, J., Németh, A., 2023. The Potential of Bacilli-Derived Biosurfactants as an Additive for Biocontrol against Alternaria alternata Plant Pathogenic Fungi.*Microorganisms11*(3), 707. DOI: 10.3390/microorganisms11030707.

Saleem, A., El-Shahir, A., 2022. Morphological and Molecular Characterization of Some*Alternaria* Species Isolated from Tomato Fruits ConcerningMycotoxin Production and Polyketide Synthase Genes. Plants 11 (1168), 1-13.DOI:org/10.3390/plants11091168.

Saleh A., Al Kamaly O., Alanazi A. S., Noman O., 2022. Phytochemical analysis and antimicrobial activity of *Rosmarinus officinalis L.* growing in Saudi Arabia : Experimental and computational approaches. *Processes,* 10 (2422), 1-8. https://doi.org/10.3390/pr10112422

Sànchez, P., Vélez-del-Burgo, A., Jorge Martínez, E.S., Postigo, I., 2022. Fungal Allergen and Mold Allergy Diagnosis: Role and Relevance of *Alternaria alternata* Alt a 1 Protein Family. Journal of Fungi8 (277), 1-11.DOI:org/10.3390/jof8030277.

Sethunga M., Ranaweera K., Gunathilake K., Munaweera I., 2022. Recent advances in the extraction methods of essential oils and oleoresins from plant materials and its potential applications. *Journal of Food and Bioprocess Engineering*, 5 (2), 151-167.

Shaban, A., 2014. Evaluation of some Fungicide Alternatives against Alternaria Fruit Rot on Tomato 42(2), 91-104

Sharifi-Rad J., Ezzat S.M., El Bishbishy M. H., Mnayer D., Sharopov F., kiliç C.S., Neagu M., Constantin C., Sharifi-Rad M., Atanassova M., Nicola S., Pignata G., Salehi B., Fokou P. V. T., Martins N., 2020. *Rosmarinus* plants : Key farm concepts towards food applications. *Phytotherapy Research*, vol (n°), 1-45.

Shiravi A., Akbari A., Mohammadi Z., Khalilian M-S., Zeinalian A., Zeinalian M., 2021. Rosemary and its protective potencies against COVID-19 and other cytoxine storm associated infections : A molecular review. *Mediterranean Journal of Nutrition and Metabolism,* 14 (2021), 401-416.

Shultz D., Rybak R., and French R. D., 2013. Alternaria Brown Spot. *Taxons A&M Agrilife Extension*, vol (n°), 1-2.

Sousa Borges, R., Sanchez Ortiz, B.L., Matias Pereira, A.C., Keita, H., Tavares Carvalho, J.C., 2018. *Rosmarinus officinalis* essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. Journal of Ethnopharmacology 229 (2019), 29-45

Stratakos, A., Koidis, A., 2016. Methods for Extracting Essential Oils: Essential Oils in Food Preservation, Flavor and Safety. Elsevier, pp. 31-38.

Stuart R. M., Bastianel M., Azevedo F. A. D., Machado M. A., 2009. Alternaria Brown Spot. *Fitopathologia*, 30 (1-2), 29-44.

Towler, G., Sinnott, R., 2013.Separation of Fluids: Chemical Engineering Design. Butterworth-Heinemann, pp. 753-806.

Verma, R., Padalia, R., Chauhan, A., Upadhyay, R., Singht, V., 2020. Productivity and essential oil composition of rosemary (Rosmarinus officinalis L.) harvested at different growth stages under the subtropical region of north India. Journal of Essential Oil Research 32(2), 144-149. DOI:10.3390/foods10112734.

Waghunde, R., Patil, R., 2010. Physiological studies of the Alternaria fruit rot (Alternaria alternata) of aonla. J. Pl. Dis. Sci 5, 73-75.

Wollinger, A., Perrin, A., Chahboun, J., Jeannot, V., Touraud, D., Kunz, W., 2016. Antioxidant activity of hydro distillation water residues from Rosmarinus officinalis L. leaves determined by DPPH assays. Comptes Rendus Chimie 19(6), 754-765.DIO:10.1016/j.crci.2015.12.014.

Yang, G., Cui, S., Ma, N., Song, Y., Ma, J., Huang, W., Zhang, Y., Xu, J., 2022.Genetic Structure and Triazole Antifungal Susceptibilities of *Alternaria alternata* from Greenhouses in Kunming, China. American Society for Microbiology (ASM) 10(3),1-18

Zaouali, Y., Bouzaine, T., Boussaid, M., 2010. Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem.Toxicol 48, 3144–3152.

Zhang M-J., Zhang X-R., Li H., Chen F-M., 2023. *Alternaria alternata*, the causal agent of a new needle blight disease on *Pinus bungeana*. *Journal of Fungi*, 9 (71), 1-12. https://doi.org/10.3390/jof9010071

Zhang, M., Zheng, X., Li, H., Chen, F., 2023. Alternaria alternata, the Causal Agent of a New Needle Blight Disease on Pinus bungeana. Funj 9(1), 71. DOI:10.3390/jof9010071

Annexe

Culture media

PDA (Potatos Dextrose Agar)

Distilled water…………………………………….…...1000ml

Potato filtrate………………………...………………..200g

D-Glucose…………………………….……………20g

So that…………………………………………….……20g

pH 4.5

Dissertation presented fo obtaining the Master's degree

Sector : Biotechnology
Speciality : *Mycology and fungal biotechnology*

Title
The antifungal Activity of Essential Oils of *Thymus vulgaris L.* and *Rosmarinus officinalis L.* against *Alternaria alternata*

Abstract

The present thesis aims to study the antifungal activity of essential oils (EOs) from the aromatic plants Thyme (Thymus vulgaris L.) and Rosemary (Rosmarinus officinalis L.) against the phytopathogenic fungus Alternaria alternata. This fungus is a ubiquitous phytopathogen capable of causing diseases to several agricultural crops and plants of economic interest. In the present work, two research investigations based on the evaluation of the effectiveness of both T. vulgaris and R. officinalis EOs against the phytopathogenic fungus A. alternata, were studied. The antifungal power of the T. vulgaris and R. officinalis EOs was evaluated at concentrations of 50 and 100µg/ml on PDA medium, and the percentage of mycelial growth reduction was calculated. In the second investigation, the minimum inhibitory concentration (MIC) of both plant EOs was tested at different concentrations (from 0 to 12 µg/ml). The obtained results from the first investigation showed that T. vulgaris EO showed the highest percentage of fungal growth reduction (53.4 and 74.9% at concentration of 50 and 100µg/100 ml, respectively) after 7 days of incubation. However, the results of MICdetermined in the second studied investigation, showed that R. officinalis EO was the most effective against the pathogen A. alternata, with a MIC value of 5000 µg/ml.
In conclusion, we can say that the studied EOs of T. vulgaris and R. officinalis have a strong inhibitory effect against the phytopathogenic fungus A. alternata. Hence, they can be considered as natural antifungal agents for controlling plant diseases caused by A. alternata. Keywords: Phytopathogenic fungi, Alternaria alternata, Antifungal activity, Essential oils (EOs), Thymus vulgaris, Rosmarinus officinalis.

Keywords : Phytopathogenic fungi, *Alternaria alternata*, Antifungal activity, Essential oils (Eos), *Thymus vulgaris*, *Rosmarinus officinalis*.

Jury Members :
Supervisor : LABBANI Fatima-Zohra Kenza MCA, ENS Assia-Djebar-Constantine
Chair of the jury : LEGHLIMI Hind MCA, UFM-Constantine

Examiner : BENOUCHENE Djamila MCA, ENSB-Constantine

Presented by : AISSAOUI Ikram – BERGUELLAH Nesrine- BOUTI Amani

Academic Year : 2022 -2023