People's Democratic Republic of Algeria
وزارة التعليم العالي و البحث العلمي
Ministry of Higher Education and Scientific Research
جامعة أبو بكر بلقايد– تلمسان
University of Abou Bekr Belkaid–Tlemcen
كلية علوم الطبيعة والحياة، وعلوم الأرض والكون
Faculty of Natural and Life Sciences, Earth and Universe Sciences
Department of Forest Resources

Laboratory No. 31: Conservation Management of Water, Soil and Forests, and Sustainable Development of Mountain Areas in the Tlemcen Region

Thesis

Presented by

Belaribi Rania

In partial ful fillment of there quirements for the

MASTER degree

In Forestry/ **Specialization:** Forest Protection

TOPIC

Diversity of the mycoflora associated with holm oak (*Quercus ilex* L.) in the Hafir forest (Tlemcen, NorthWestern Algeria).

Defended on June12, 2025, before the jury composed of:

President	Haddouche Driss	Prof.	University of Tlemcen
Examiner	Kholkhal Djamel	MCB	University of Tlemcen
Supervisor	Smahi Chouikhi Hadjer	MCA	University of Tlemcen

Academic year 2024/2025

Dedication

To my younger Rania this victory is yours; you dreamed it long before I believed it, and every tear you buried in silence, I've forged into a crown only the dreamers could wear.

To my dad and mom,

Who gave me the opportunities they never had.

You are the roots from which I've grown, your endless patience, and your unconditional love have paved every step of this journey. In moments of doubt, it was your faith that lifted me, in moment of struggle your prayers that carried me through. This achievement belongs to you as much as it belongs to me thank you for giving me everything without ever asking for anything in return. Thank you for being my greatest gift.

To my dear siblings: Dounia, Doaa, and Aymen.

You are my safe haven and my constant joy. In your laughter, I found comfort. In your words, I found encouragement. And in your love, I found the strength to rise. No matter how far I go, your presence walks beside me always.

And to my brother Imad,

Every success I achieve in this life is yours before it is mine. You may be gone from this world, but never from my heart. I carry you with me in every step, every dream, and every silent victory because even in your absence, you continue to walk beside me.

Each of you, in your own way, reminded me why I must keep going "Always and forever"

Acknowledgements

In the name of Allah, the Most Gracious, the Most Merciful.

All praise is due to Allah who granted me the strength, patience, and perseverance to complete this work. Peace and blessings be upon our beloved Prophet Muhammad, the light of guidance and mercy to all mankind.

I would like to express my profound gratitude to all those who have supported me, guided me, and believed in me throughout this academic journey and especially during the preparation of this graduation thesis.

First and foremost, I would like to express my deepest appreciation to my supervisor, Doctor ***Smahi Chouikhi Hadjer***, for her exceptional guidance, patience, and unwavering support. Her encouragement and trust throughout the research process inspired me to do my best. And her insightful feedback was fundamental in refining this work.

I would like to express my sincere gratitude to ***Prof. HADDOUCHE Driss*** and ***Dr. Kholkhal Djamel*** for kindly accepting to be part of the jury. Their valuable time, expertise, and insights are deeply appreciated and contribute greatly to the quality of this academic work.

I would also like to extend my sincere thanks to ***Prof. Bouharaoua Rachid Tarik***, whose valuable assistance and academic advice have significantly contributed to this thesis. His readiness to help and his deep knowledge were truly appreciated during the most critical stages of my research.

I sincerely thank ***Mrs. Amrani Nezha***, engineer at Laboratory "N°3 Mycology" for her invaluable support during the experimental work,

I would also like to pay tribute to the Director of Laboratory No. 31 – Management and Conservation of Water, Soil, and Forests, ***Prof. Mostefai Noureddine***, as well as his team, in particular ***Mrs. MAAZOUZ-BENFRIHA Sarra*** and ***Mr. Laidouzi Mohamed*** .

To my dear friend ***Sghir Narjes KHadidja***, thank you for your sincere friendship, kind presence, and emotional support. Your encouragement and warmth made this journey lighter and more meaningful.

I am deeply grateful to all the professors, lecturers, and staff of the **Department of Forestry Ressources** for providing a strong academic foundation and for their inspiring commitment to education and excellence. I also extend my heartfelt thanks to my friends and colleagues for their unwavering support, shared moments of stress and laughter, and true companionship throughout this journey.

May Allah reward you all abundantly and guide us all toward continued success and fulfillment.

ملخص: تنوع الفطريات المرتبطة بالبلوط الأخضر(*Quercus ilex*) في غابة أحفير (تلمسان، شمال غرب الجزائر)

أُجريت هذه الدراسة في غابة أحفير (تلمسان) بهدف تقييم الحالة الصحية للغطاء الغابي وتحديد الفطريات المرتبطة بظاهرة تدهور الأشجار. تم أخذ عينات من ثلاثين شجرة تظهر عليها أعراض مرضية (مثل تغير لون الأوراق، والجفاف، وظهور التقرحات)، ثم تم تحليلها في المختبر. وقد تم تحديد الفطريات المعزولة من خلال الملاحظات المجهرية والعيانية.

في المجموع، تم عزل عشر أنواع فطرية، من بينها *Dothiorella iberica* (20%)، *Alternaria alternata* (19%) و*Penicillium sp.* (17%). يشير مؤشر تنوع شانون (H’ = 2.11) إلى وجود تنوع فطري معتدل إلى مرتفع. وقد تبيّن أن الأنواع التابعة لعائلة *Botryosphaeriaceae* مرتبطة بشكل رئيسي بأعراض التدهور، لا سيما لدى أشجار *Quercus ilex* و*Quercus suber*. تؤكد هذه النتائج تورط فطريات ممرضة انتهازية في تدهور الأشجار المدروسة. كما أظهر اختبار التضاد الحيوي في الزراعة المخبرية التأثير المثبط للفطر *Trichoderma sp.* على *Diplodia sp.*، مما يشير إلى إمكانية استخدامه في المكافحة الحيوية.

الكلمات المفتاحية: غابة أحفير، فطريات، ممرض نباتي، *Quercus ilex*، Botryosphaeriaceae

Résumé : Diversité de la mycoflore associé au chêne vert (*Quercus ilex*) dans la forêt de Hafir (Tlemcen Nord-ouest Algérien).

La présente étude a été menée dans la forêt de Hafir (Tlemcen) afin d’évaluer l’état sanitaire des peuplements forestiers et d’identifier les champignons associés au dépérissement. Trente arbres présentant des symptômes (décoloration, dessèchement, chancres) ont été échantillonnés, puis analysés en laboratoire. Les isolats ont été identifiés par observations macro- et microscopiques.
Au total, dix espèces fongiques ont été isolées, dont *Dothiorella iberica* (20 %), *Alternaria alternata* (19 %) et *Penicillium sp.* (17 %). L’indice de Shannon (H’ = 2,11) révèle une diversité modérée à élevée. Les espèces de la famille des Botryosphaeriaceae ont été principalement associées aux symptômes de dépérissement, notamment chez *Quercus ilex* et *Quercus suber*. Ces résultats confirment l’implication de champignons pathogènes opportunistes dans le déclin des arbres étudiés. Un test d’antagonisme in vitro a montré l’effet inhibiteur de *Trichoderma sp.* sur *Diplodia sp.,* suggérant un potentiel de biocontrôle.

Mots clés : Forêt de Hafir- champignons- phytopathogène- *Quercus ilex*- Botryosphaeriacea.

Abstract: Diversity of the mycoflora associated with holm oak (*Quercus ilex*) in the Hafir forest (Tlemcen, northwestern Algeria).

This study was conducted in the Hafir Forest (Tlemcen) to assess the health status of forest stands and to identify fungi associated with tree decline. Thirty symptomatic trees (showing discoloration, drying, and cankers) were sampled and analyzed in the laboratory. The isolates were identified based on macroscopic and microscopic observations.

In total, ten fungal species were isolated, including *Dothiorella iberica* (20%), *Alternaria alternata* (19%), and *Penicillium sp.* (17%). The Shannon diversity index (H’ = 2.11) indicates a moderate to high level of fungal diversity. Species belonging to the Botryosphaeriaceae family were mainly associated with decline symptoms, particularly in *Quercus ilex* and *Quercus suber*. These findings confirm the involvement of opportunistic pathogenic fungi in the decline of the studied trees. An in vitro antagonism test revealed the inhibitory effect of *Trichoderma sp.* on *Diplodia sp*., suggesting a potential for biological control.

Keywords: Hafir Forest – Phytopathogenic- Fungi- *Quercus ilex*- Botryosphaeriacea.

Abbreviation and acronyms

DNA: Deoxyribonucleic Acid
C°: Degrees Celsius
D.G.F: General Directorate of Forests
DSF: Statistical and Fiscal Declarations
IPCC:Intergovernmental Panel on Climate Change
g: Gram
h: Hour
ha: Hectare
km: Kilometer
L: Liter
m: Meter
m^2: Square meters
mm: millimeters
mm^2: Square millimeters
ml: Milliliter
log: Logarithm
mm/s: Millimeters per second
MEA: Malt Extract Agar
PDA: Potato Dextrose Agar
AA: Agar-Agar
MAT: The mating-type loci
LAI: The Leaf Area Index
Sp: Species
T°: Temperature
µm: Micrometer

Abbreviation and acronyms

H': The Shannon diversity index

%: Percent

B.P.: BeforePresent

m.a.s.l: Meters Above Sea Level

Table of content

Abbreviation and acronyms

List of figures

List of tables

General introduction

List offigures

List of tables

General introduction

Forest ecosystems are complex and vital components of the environment, delivering key services such as carbon storage, soil and water regulation, and biodiversity conservation. In the Mediterranean region, forests also hold important socio-economic and cultural value, supporting traditional land use and hosting endemic species (Quézel & Médail, 2003). However, they are increasingly threatened by climate change, land degradation, and emerging diseases, particularly those caused by pathogenic organisms (FAO, 2020; IPCC, 2021). Among these, cryptogamic diseases mainly caused by spore-producing organisms like fungi pose a serious risk to forest health. Phytopathogenic fungi, in particular, can impair tree vitality and forest regeneration (Desprez-Loustau *et al.,* 2006; Slippers & Wingfield, 2007). Despite their impact, such pathogens remain understudied, especially in Mediterranean forests, where symptoms may be subtle or mistaken for abiotic stress (Sánchez *et al.,* 2015).

The Algerian Forest ecosystem, part of the broader Mediterranean biome, is known for its rich biodiversity but is increasingly vulnerable to degradation due to anthropogenic pressures and climatic extremes (Ikermoud, 2000; Guit *et al.,* 2016).

Within this context, Hafir Forest, located in the Tlemcen National Park in northwestern Algeria, has shown alarming signs of tree decline. This forest is primarily composed of Holm oak (*Quercus ilex*) and Cork oak (*Quercus suber*), along with other species such as Aleppo pine (*Pinus halepensis*) and Cade juniper (*Juniperus oxycedrus*).

Cryptogamic diseases mainly caused by pathogenic fungi, oomycetes, and other spore-producing organisms pose a significant threat to forest health and sustainability. These pathogens are responsible for various symptoms, including foliar necrosis, wilting, cankers, root rot, and dieback, which can ultimately lead to the decline or death of affected trees. Their impact extends beyond individual trees, disrupting entire forest ecosystems by altering biodiversity, nutrient cycling, and soil structure.

In recent years, symptoms such as leaf yellowing, wilting, dieback, and overall tree decline have been increasingly observed in the Hafir Forest. These symptoms, affecting both aerial and subterranean parts of the trees, strongly indicate the involvement of cryptogamic pathogens.

This thesis aims to explore the phytopathogenic fungi associated with tree decline in Hafir Forest. The study is driven by the need to better understand the biological factors underlying the observed symptoms and to assess the potential risks that fungal pathogens may pose to the long-term health and stability of the forest ecosystem. Specifically, this work seeks to:

- Identify and characterize the fungal species isolated from symptomatic trees;
- Assess the antagonistic properties of selected isolates against identified pathogens.

To address these objectives, the thesis is organized into two main parts.
The **first part** provides a comprehensive review of cryptogamic diseases, with a particular

focus on those caused by phytopathogenic fungi affecting forest ecosystems. It also presents an overview of the study area and discusses the ecological and biological characteristics of Holm oak (*Quercus ilex*), one of the primary host species. Well-documented cases of forest decline in the Mediterranean basin are included to offer broader context.

The second part comprises the experimental component, including the Materials and Methods section, followed by the Results and Discussion. The thesis concludes with a general conclusion and perspectives for future research.

Chapter one :

Cryptogamic diseases

I.1. Introduction

The interaction between pathogens and their hosts is based on a complex and precise molecular dialogue, where each side plays a decisive role in determining the outcomewhether susceptibility or resistance. A deeper understanding of these interactions opens up promising new avenues for the development of innovative and effective disease control strategies.

As George Massee said “Knowledge is power, and the individual with the most extensive understanding of their specialized field is the one most likely to succeed”. Hence it follows that knowledge of the broad principles regulating the mode of life of those groups of parasites which prove so injurious to trees and cultivated plants should be clearly grasped.

A plant disease is defined as “any malfunctioning of host cells and tissues caused by ongoing exposure to a pathogenic agent or environmental factor, leading to the development of symptoms (Agrios, 2005). Three interrelated elements result in disease: a susceptible host plant, a virulent pathogen, and a favorable environment or climate. These factors are commonly represented as the disease triangle.

Forest pathogens include fungi, oomycetes, bacteria, phytoplasmas, parasitic plants, viruses, and nematodes. Native forest pathogens, which are important parts of many forest ecosystems (Hansen & Goheen, 2000), play a key role in shaping species richness and abundance, as well as influencing forest succession, structure, and composition at both the stand and landscape levels (Winder & Shamoun, 2006).They also have a significant impact on animal populations, nutrient and water cycling, and the overall functioning of the ecosystem. Native forest pathogens sometimes conflict with management objectives, as they may harm valuable forest resources.

Forest decline diseases are complex and result from a combination of interacting factors (Manion, 1991; Manion & Lachance, 1992).

Predisposing factors are long-term or slowly changing conditions, such as soil moisture, stand density, and precipitationthat make trees more susceptible to disease. Inciting factors are short-term elements, such as defoliating insects or drought, which cause acute stress. While trees affected solely by inciting factors may recover quickly, recovery is significantly slower when predisposing factors are also present. Contributing factors are typically secondary pathogens or insects that ultimately kill trees already weakened by both predisposing and inciting factors.

Pathogens, ubiquitous across forest ecosystems, play a pivotal role in shaping forest dynamics, primarily through their capacity to induce tree mortality. Their impact can manifest at multiple spatial scalesfrom extensive landscapes and regions to individual trees or small tree clusters. The resulting mortality may lead to profound alterations in forest structure, species composition, and growth dynamics, thereby influencing broader ecological processes, including biodiversity maintenance and overall ecosystem functioning.

Although often perceived as harmful, pathogens also play essential ecological roles. They contribute to the maintenance of plant species diversity, facilitate succession processes, and enhance the genetic diversity and population structure of host species. Additionally, forest pathogens are commonly regulated by other organisms, which helps prevent uncontrolled proliferation and ecosystem imbalance.

Fungi, as one of the most prominent groups of plant pathogens with substantial ecological impact, will be examined first.

A critical aspect of their life history that must be understood to explain their pathogenic behavior is their nutritional strategy. It is this feature that underlies their ability to infect, colonize, and often kill host plants, including trees, leading to considerable ecological and economic consequences.

It is widely recognized that trees and flowering plants obtain their nourishment from two primary sources: a portion comes from the soil, and another from the air. These substances are inorganic, meaning they are not derived from living organisms. However, with the help of chlorophyll, the green pigment found in leaves, these inorganic substances are transformed into organic molecules that serve as the plant's food, the direct result of the metabolic activity of living green leaves.

In contrast, fungi lack chlorophyll entirely, which means they are incapable of assimilating inorganic nutrients through photosynthesis. Instead, they depend on organic matter, which they obtain by decomposing and feeding on the remains of other plants. For this reason, fungi are frequently found colonizing areas where trees have declined or died.

As a clear example of the significant impact fungi can have on forest ecosystems, the degradation of cork oak forests in Algeria(Bouhraoua*et al.,* 2015), particularly in the Hafir Forest(Boudy, 1950; Bentouat&Bouhraoua, 2009), was vital for both ecological balance and the economy. However, these forests experienced significant degradation over time due to several factors. These factors were classified into three categories: predisposing factors (such as poor forest management), inducing factors (like climate change), and accelerating factors, which are primarily fires, insect attacks (defoliators, xylophages, and xylomycetophages) such as *Zeuzeyrapyrina*, *Lymantria dispar*, and *Platypus cylindrus*(Delb& Hein, 2001; Battisti *et al.,* 2015), along with fungi that played a key role in the decline, including species like *Phytophthora cinnamomi*, *Botryosphaeria spp*. (Smahi*et al.,* 2017a, 2017b), and *Armillaria mellea*, which cause root rot, wood decay, and weakening of the trees. Additionally, pathogens such as *Pythium spiculum* and *P. sterilum* (Bendjebbar*et al.,* 2023) contributed to the degradation.

I.2. History of mycology

The Kingdom Fungi, home to molds, mushrooms, lichens, rusts, smuts, and yeasts, consists of eukaryotic organisms with highly diverse life histories. These organisms play vital roles in the biosphere, contributing to ecosystems, human industry, medicine, and scientific research (Jason*et al.,* 2009). They are considered an ancient group, but not as old as bacteria, which may be 3.5 billion years old, according to fossil data. The earliest fossils are from the Ordovician period, 460 to 455 million years ago (Lori *et al.,* 2012).

The Mycenaean civilization, one of the greatest ancient civilizations, may owe its name to a legendary mushroom. According to Greek mythology, the hero Perseus, while thirsty, came across a mushroom (mykes) and drank the water flowing from it. Pleased by this discovery, he named the place Mycenae after the mushroom (Graves, 1955).

The term mycology is etymologically derived from the Greek words *mykes* (mushroom) and *logos* (discourse), meaning the study of fungi (Deacon, 2005). The invention of the microscope by Anton van Leeuwenhoek in the 17th century marked the beginning of detailed fungal research. The man who deserves the honor of being called the founder of the science of mycology is Antonio Micheli, an Italian botanist, with his publication of *Nova Plantarum Genera* in 1729, a pioneering work on fungi (Micheli, 1729; Ainsworth, 1976). Later, in 1753, Carl Linnaeus included fungi under the cryptogams in his book *Species Plantarum*; referring to them as cryptogamic fungi, further advancing the field (Linnaeus, 1753; Ainsworth, 1976).

The year of 1588 saw a landmark in the development of mycology: the publication of the first observation of fungal spores, by ***Giambattista della Porta*** who was both precocious and versatile (Ainsworth, 1976).

I.3. What are fungi?

As pathogens, symbionts, and decomposers, fungi are a varied group of eukaryotic organisms that play crucial roles in ecosystems.

After being long classified as plants, true fungi, or eumycetes, now form a separate kingdom: the fungal kingdom. These organisms share several characteristics as eukaryotes, using their cell walls to absorb nutrients and digest food externally (Carris *et al.,* 2012).

The majority of fungi have a body (thallus) made up of tiny tubular cells called hyphae, and they reproduce by spores. As heterotrophs, fungi get their energy and carbon from other living organisms, much like animals do (Popier, 1985).

Some fungi, known as biotrophs, obtain their nutrition from living hosts, such as plants or animals, while saprotrophs, also known as saprophytes or saprobes, derive their nutrients from dead plants or animals. Necrotrophs are fungi that infect living hosts but kill the host cells to obtain their resources(Alexopoulos*et al.,*1996).

They interact with other organisms through mutualism, commensalism, and, unfortunately, parasitism. (Gomdola, 2022).

Globally, an estimated 1.5 million fungal species exist, but as of 2011 (book), only around 80,000 species had been identified as plant pathogens, and 200 were known to infect humans and animals. However, by 2022, with advancements in science and increased research in mycology, it was estimated that around 270,000 fungal species are associated with plants, and 325 species have been confirmed to infect humans (Jampilek, 2022).

In forests, fungi can pose significant threats to ecosystems, leading to the destruction of vast woodland areas. For example, oak forests have experienced morbidity and mortality attributed to canker-causing taxa such as *Diplodia corticola* and *Disculaquercina* (current name: *Dendrostomaleiphaemia*) (Gomdola, 2022).

However, fungi can alter their way of life under favorable or unfavorable conditions. Some endophytes that colonize plants asymptomatically can become pathogenic, and vice versa, under certain conditions (Bhunjun, 2023).

For instance, *Sphaeropsissapinea* can cause disease when plants are stressed under conditions such as drought, extreme temperatures, or mechanical wounds (Gomdola, 2022). Furthermore, *Phytophthora cinnamomi* infects tree roots and thrives under waterlogged soil conditions, leading to root rot and dieback in various forest species (Hardham, 2005).

Plant diseases and their intensity arise from the interaction of three key factors: the host plant, the pathogen, and the environmental conditions. This relationship is commonly illustrated through the disease triangle model (Tjosvold, 2018) (Fig. 1).

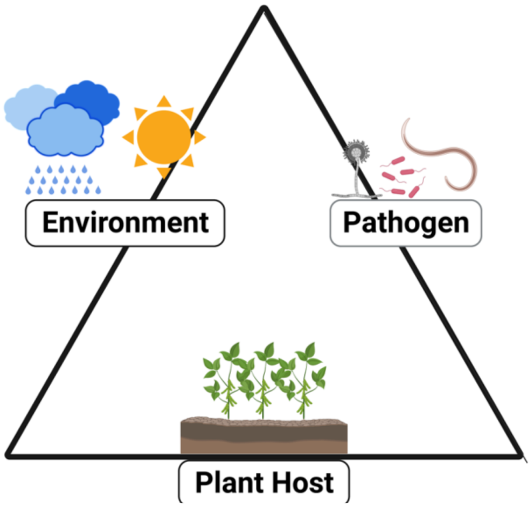

Figure 01. The disease triangle (Leo Rocha, 2023).

I.4. Morphological characteristics of fungi

A better understanding of the fungal lifestyle, cell biology, metabolism, and morphogenesis is essential to understand their effect on plants and forest ecosystems in particular:

I.4.1. Mycelium

A vital characteristic of fungi that has enabled them to effectively exploit a variety of ecological niches is the development of a filamentous thallus known as the mycelium.

Mycelium is composed of branching, microscopic tubular cells called ***"hyphae"*** that grow through and across substrates or food sources, secreting enzymes that break down complex substrates into simple compounds that can be absorbed through the cell wall (Carris *et al.,* 2012) (Fig. 2).

The fungal cell wall in the kingdom **Fungi** is a dynamic organelle that functions in several important processes. It is composed of chitin, a long linear homopolymer of beta-1,4-linked N-acetylglucosamine, which is considered to be relatively minor yet structurally important. Glucan is the major structural polysaccharide of the fungal cell wall, constituting approximately 50-60% of the wall by dry weight, along with chitosan and other components (Bowman &Free, 2006).

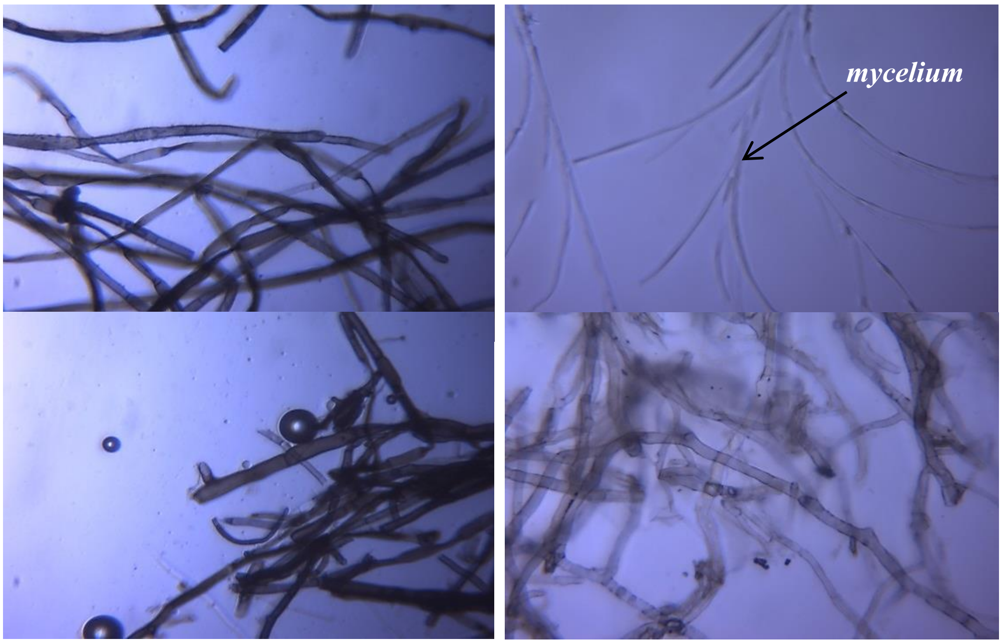

Figure 02. Morphology of mycelium (Original).

I.4.2. The hyphae

The hypha, a cylindrical cell with a distinctive tip that resembles a hemi-ellipsoid in shape but may be more precisely defined mathematically by the hyphoid equation, is the vegetative unit of growth in filamentous fungi (Riquelme, 2018).

Hyphae develop from a germinating spore or other types of propagules, extend outward from the point of establishment, and lengthen virtually exclusively at the tips (Carris *et al.,* 2012). Due to apical development, hyphae are relatively uniform in diameter, and mycelium that develops freely forms a circular colony on solid substrates that support fungal growth.

I.4.3. Spores

The most common fungal survival strategy is the production of spores. They spread not just by wind and water, but also by other species. Earthworms, for example, disperse fungi by mixing soil layers, and fungal spores can also stick to an insect's surface, generally through hydrophobic interactions.

Many different types of fungal spores can be distinguished, which are formed either asexually (conidia) or sexually (Timon *et al.,* 2013; Blancard, 2015) (Fig. 3).

Asexual spores are formed after mitosis (mitospores) without the involvement of meiosis. These spores can be produced within specialized structures called **"*conidiomata*"**, which resemble fruiting bodies typically associated with the formation of sexual spores. While conidiomata formation is widespread among various fungal genera, it was initially assigned to a specific group called the coelomycetes, which includes species such as *Phoma* and *Colletotrichum* (Timon *et al.,* 2013). Sexual reproduction is a common process across the fungal kingdom, and the spores produced during this process are categorized into different types. Zygospores are sexual spores produced by *Zygomycota* in zygosporangia. The diploid motile zoospores are the result of the complex sexual reproduction of *Chytridiomycota*, while the basidiospores of *Basidiomycota* and the ascospores of *Ascomycota* are produced on basidia and within asci, respectively (Lee*et al.,* 2010).

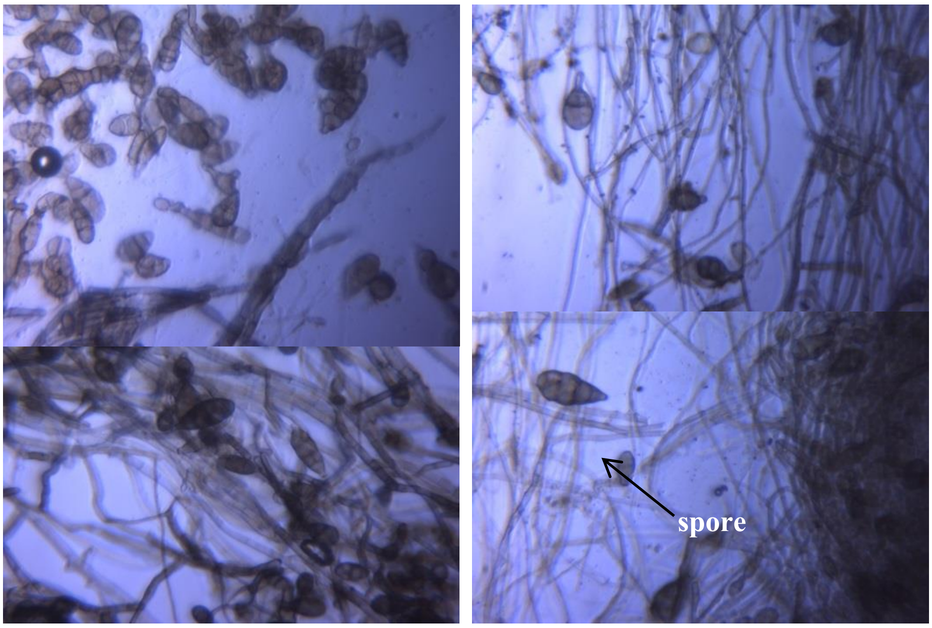

Figure 03. Morphology of Spores (Original).

I.5. Fungi life cycles

Fungi represent a wide range of organisms characterized by significant variation in their reproductive strategies. While almost all species can reproduce sexually, many reproduce asexually for the majority of their life cycle (Bart *et al.,* 2016).

I.5.1. Asexual reproduction

According to Louis *et al.* (2010), asexual reproduction serves as the main method of dispersal for many pathogens, especially within ascomycetes. This process occasionally occurs through the fragmentation of the thallus, but more commonly, it takes place through budding (sporulation).

Fungi do not fuse the nuclei of two cells to reproduce asexually. When the mycelium breaks down into pieces known as arthrospores, the simplest form of asexual reproduction takes place. Another form involves the production of independent germ spores, known as conidia, which are produced by specialized hyphae called conidiophores.

Conidia vary in shape, size, and color, and can be either unicellular or multicellular. Thick-walled spores produced from mycelial cells, known as chlamydospores, serve as resting spores. These spores allow fungi to survive prolonged unfavorable conditions, such as extreme temperatures or drought (Shubhrata, 2005).

The cells that give rise to asexual spores are typically referred to as conidiogenous cells. In classifying the conidial stages of fungi, many species that reproduce asexually are classified as imperfect fungi.

Fungal reproduction is heavily influenced by the formation and generation of asexual spores. Fungi produce spores in such vast numbers that they are present in the atmosphere and throughout various parts of the biosphere. These spores are widespread and possess an extraordinary ability to endure harsh conditions, enabling them to remain dormant. This overproduction of spores serves as a vital survival mechanism, ensuring the species' ongoing existence (Benny *et al.,* 2001).

I.5.2. Sexual reproduction

There appear to be several disadvantages to sexual reproduction. First of all, compared to asexual reproduction, it requires more time and effort. An example of this would be the time spent finding a mate. Furthermore, asexual reproduction has an advantage because it is less costly to produce male structures in a sexual reproductive system (Mohlmann, 2011).

Sexual reproduction in fungi occurs in three stages. First, haploid cells of compatible mating types fuse (plasmogamy). This is followed by the fusion of the two haploid nuclei (karyogamy). In response to nutritional limitations, the newly generated diploid cell may undergo meiosis to produce haploid cells. Similar to how two sperm or two eggs do not naturally fuse to create a zygote, the initial fusion of haploid cells requires that the cells be of compatible (often opposite) mating types. The genetic material at the mating-type locus determines the mating type of a given cell (Wallen & Michael, 2018) (Fig. 4).

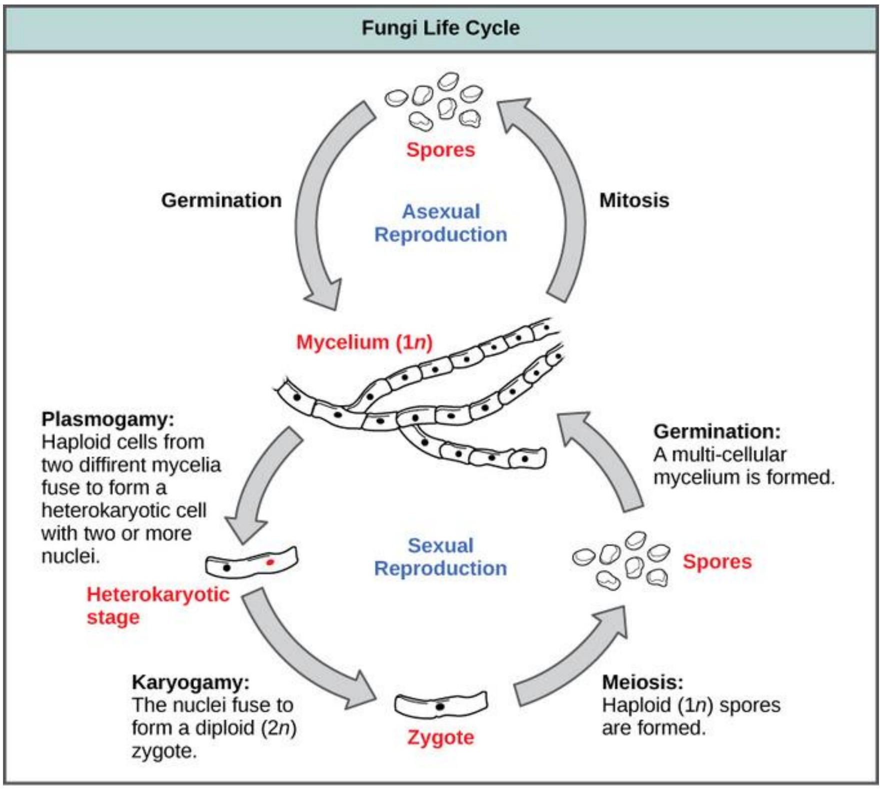

Figure 04. Fungal life cycle: Asexual and sexual reproduction pathways (Libertexts, 2024).

I.5.2.1. The Ascomycota

The phylum *Ascomycota*, also known as the sac fungi, includes a wide range of organisms such as plant, insect, and mammalian pathogens, as well as unicellular yeasts, saprotrophs, and mutualistic symbionts. These fungi produce meiotic spores within a specialized sac-like structure called an ascus (Wallen & Michael, 2018).

The mating-type loci (MAT), which encode key transcription factor genes involved in the regulation of sexual reproduction and speciation, play a crucial role in the sexual cycle of ascomycetes. During this process, two compatible partners fuse their genetic material through recombination or crossing-over, followed by meiosis and mitosis, resulting in genetically diverse offspring.

Sexual structures such as asci and ascospores are found within fruiting bodies. When released, ascospores that land on a suitable substrate germinate and develop into male (+) and female (–) mycelia, representing the two mating types. The male mycelium forms an antheridium, while the female mycelium develops an ascogonium, distinct reproductive organs involved in sexual reproduction (Indunil*et al.,* 2022).

Ascomycetes frequently undergo sexual reproduction in response to unfavourable environmental conditions, leading to a high degree of genetic diversity among species. This mode of reproduction not only favors the selection of beneficial mutations that support adaptation to changing environments, but also contributes to the elimination of harmful mutations (Bart *et al.,* 2016).

I.5.2.2.The Basidiomycetes

Canonical sexual reproduction in basidiomycete fungi involves the fusion of two haploid individuals of different mating types, resulting in a heterokaryotic mycelium composed of genetically distinct nuclei (Yen-Wen Wang *et al.,* 2023).

In basidiomycetes, the sexual cycle begins with the fusion of genetically distinct homokaryotic hyphae or haploid yeast cells to form a dikaryon. In this stage, the two haploid parental nuclei coexist and divide without fusing during hyphal elongation, typically via clamp connections, which are hook-like structures that ensure the proper distribution of the two genetically distinct nuclei during mitosis (Marco *et al.,* 2018).

Nuclear fusion (karyogamy) occurs within basidia or other specialized structures such as teliospores, followed by meiosis to produce haploid basidiospores (meiospores), thereby completing the life cycle (Marco *et al.,* 2018).

I.6. Fungal infection processes

The infection process of pathogenic fungi in plants entails a highly coordinated, multi-phase sequence comprising adhesion to the host surface, penetration of plant tissues, subsequent colonization, and eventual proliferation within host structures. These stages are orchestrated through sophisticated molecular and structural strategies that facilitate successful host invasion and disease establishment (Li *et al.,* 2022).

I.6.1. Stages of fungal infection

The process of fungal infection in plants begins with the adhesion of spores or hyphae to the plant surface(Idnurm & Howlett, 2001), which is crucial for the pathogen to start colonizing. After adhesion, fungi form specialized structures like appressoria to penetrate plant tissues by generating mechanical pressure. Fungal pathogens weaken plant defenses by secreting enzymes that degrade the cuticle and cell wall, or by entering through natural openings such as stomata or wounds. Once inside, the fungus establishes a parasitic relationship, absorbs nutrients, and spreads through the plant's vascular system.

I.6.2. Pathogenic growth strategies

- **Biotrophic growth:** As biotrophs, some fungi extract nutrients from living host cells without causing immediate harm. Specialized structures, such as haustoria, are often formed during this type of infection.
- **Necrotrophic growth:** These fungi often produce toxins, kill host cells, and feed on the decomposing tissue.

- **Hemibiotrophic growth:** Fungi employing both strategies, initially acting as biotrophs and later switching to necrotrophic growth.

I.7. Factors influencing the spread and severity of fungal pathogens in forest ecosystems

The capacity of pathogens to spread disease to their hosts is determined by a variety of factors. Newly discovered pathogens are responsible for much of the global increase in plant diseases. When these pathogens emerge, they can spread to other geographical areas and affect additional hosts. They are capable of switching between different lifestyles depending on favorable or unfavorable conditions. For example, *Diplodia mutila*, an endophyte of *Iriarteadeltoidea*, can become pathogenic under excessive light conditions (Gomdola*et al.,* 2020).

I.7.1. Effects of host variability, mobility and infectiousness on the spread offorest diseases

The ability of fungal pathogens to affect forest trees can be influenced by several factors. These include their resilient nature, their ability to survive without a host or favorable conditions, their mode of propagation, and their ability to adapt to different environments.

Among the most critical factors affecting the spread and invasive capacity of aggressive forest infections is the reproductive system. This factor is important for its ability to generate more virulent strains and to facilitate adaptation to new host species in different environments. The type of reproduction also plays an important role, as the rate of asexual reproduction can influence the invasion dynamics of an introduced population (Prospero & Cleary, 2017).

Moreover, host susceptibility varies depending on several factors, including local site conditions, genetic diversity, and physical characteristics (Jules *et al.,* 2014). Invasion can also be facilitated by hybridization between two exotic species or between exotic and interfertile native species (Gonthier &Garbelotto, 2013; Langwig*et al.,* 2021).

I.7.2. Environmental factors and climat change

Environmental conditions, particularly temperature, humidity/soil moisture, and light intensity, can lead to variations in pathogen virulence as well as in the susceptibility of host plants (Yu Liu &Fangliang He, 2019).

Climate change is expected to impact biotic disturbances, including the frequency and severity of pest and disease outbreaks (Porta *et al.,* 2008). According to Raitelaitė*et al.* (2016), coniferous trees, due to their long lifespan, are particularly vulnerable to these changes as they are unable to adapt quickly to shifting environmental conditions (Tab. 1).

Events such as floods, storms, and hurricanes can promote the emergence of more virulent pathogen strains and expand the geographical range of pathogens or their vectors, allowing diseases to spread into areas where they were previously unreported.

For instance, *Phytophthora cinnamomi*, which is sensitive to frost, tends to migrate toward warmer regions where it infects new hosts (Smahi, 2019).

Table1. The impact of climate on tree pathogens in northen Europe (Brodde *et al.,* 2016; Hietala *et al.,* 2013).

Pathogen	Region	Results	Climate Influence
Hymenoscyphusfraxineus	Norway	Monitoring showed that higher summer temperatures increased the infection pressure of H, fraxineus, while competition with H, albidus was studied	Summer temperatures favored growth of *H.fraxineus* populations, with exponential growth during favorable years.
Diplodiatip blight	Sweden	The first large outbreak in northern Europe occurred in 2016, Isolated attacks started 10 years earlier, reaching 90% of trees.	Warm temperatures caused greater damage and slow growth. Cold and rainy conditions supported growth and impaired attacks.

I.7.3. Biological factors

Phytopathogenic fungi produce toxins that can play a key role in the development of plant diseases. These toxins, considered part of the pathogen's metabolism, are non-enzymatic chemical compounds that are toxic to plants. They can induce specific symptoms such as wilting, growth inhibition, chlorosis, necrosis, and leaf spotting. Even at very low concentrations, these toxins can disrupt the normal physiological functions of plants (Peng *et al.,* 2021).

In addition, fungi can spread through various biological mechanisms. The dispersal of fungal spores involves interactions with both abiotic and biotic factors, including: wind and water (Sándor, 2020), soil organisms (Dijksterhuis, 2013), and insects (Belhoucine, 2008; Wösten & Dijksterhuis, 2013).

I.8. Symptoms offungaldiseases

Wood pathogens induce symptoms such as cankers, gummosis, wood decay, blight, and dieback. In severe cases, dieback of shoots, branches, and main stems can ultimately lead to tree mortality (Guarnaccia *et al.,* 2022).

I.8.1. Leaf symptoms

According to Dubey & Pandey (2017), fungal colonization can lead to a variety of symptoms such as leaf discoloration, leaf scorch, sooty spots, leaf shot-holes, tar spots, powdery mildew, black molds, downy mildew, rusts, smuts, galls, and sooty molds (Fig. 5)

Figure 05. Symptoms of fungal colonization on cork oak leaves (Original).

I.8.2. Root symptoms

Trees infected by soil fungi exhibit similar above-ground symptoms, including slow growth, yellowing of foliage, leaf or needle loss, branch dieback, poor cone production, and ultimately, tree death (Goheen & Otrosina, 1998).

I.8.3. Branch symptoms

Branch symptoms caused by fungi include bark necrosis, characterized by discoloration and lesions, branch dieback, and reduced vigor (Smahi*et al.,* 2017a) (Fig. 6).

Figure 06. Branch symptoms caused by fungal infection on cork oak trees (Original)

I.8.4. Bark and trunk symptoms

Fungal diseases affecting the bark and trunk of trees can have devastating impacts on their health and structural integrity. Fungi are often trapped in the tree's bark due to its sticky nature, which creates a favorable environment for spores to adhere and proliferate (Magyar *et al.,* 2022). Among the trunk symptoms are: epicormic shoots, bark necrosis, cankers (Fig. 7).

Figure 07. Trunk symptoms caused by fungal infection, observed on cork oak, showing epicormic shoots, bark necrosis, and cankers (Original).

Fungal attack symptoms are highly variable and closely depend on the causal agent involved. Each pathogenic fungus can cause specific manifestations, which appear as distinct signs. This symptomatic diversity reflects the complexity of interactions between the fungus and the host, as well as the different infection strategies employed by fungal agents (Tab. 2).

Table 2. Forest phytopathogenic diseases and their symptoms (Bulmensteie*t al.*, 2022; GaicheroDeclerk& Marquez, 2022; KabaktepeSevindk&Akata, 2020; Xing *et al.*, 2022).

Disease	symptoms	Pathogen
Diplodia tip blight	Shoot brownin, needle death, necrotic needle, dieback, canker on branches.	*Diplodia sapinea*
Root rot and seedling Damping off	Root rot, brown stem, lesions, leaf yellowing, wilting, plant death.	*Phytophthora cinamomi*
Powedry mildew	White powdery patches/films on leaves, stems; curling or distortion of leaves.	Erysiphales
Canker	Lesions, wilting, dieback, reduced photosynthesis, impaired transport.	*Botrosphaeria dothidea*

I.9. Strategic approaches to combat phytopathogenic diseases

Effective disease management requires the identification of symptoms associated with major diseases, an understanding of the environmental conditions that favor their development, awareness of the timing of pathogen introduction into the crop production cycle, and knowledge of effective control measures (Ogunsiji & Odusanya, 2020).

I.9.1. Genetic engineering for resistance against fungal pathogens

The adoption of genetic engineering technology for fungal resistance remains limited. However, several advancements have shown promising results. Many transgenic plants exhibiting remarkable resistance to multiple fungal pathogens have been successfully developed.

I.9.2. Biocontrol

Biological control refers to the use of microorganisms in the management of plant diseases. To combat *Rhizoctonia* in tobacco, for instance, *Trichoderma lignorum* was registered as a fungicide in 1954 under Japan’s Agricultural Chemicals Regulation Act. This marked the first documented instance of biofungicides in the world (Mekonene & Kebede, 2024).

I.9.3. Silvicultural and phytosanitary control

According to McAree (1975), silvicultural methods for controlling cryptogamic diseases include several strategies:

1. **Avoidance of thinning:** Especially on high-hazard sites. Trees may be planted at wider spacings to avoid the need for thinning, thereby reducing root contact and minimizing the risk of disease transmission.
2. **Thinning at the right time:** If thinning is necessary, it should be carried out during periods that are unfavorable for fungal development, such as warmer seasons when elevated stump-top temperatures can deactivate pathogens.
3. **Stump removal:** In certain cases, stumps are removed to decrease the amount of available inoculum. This method is particularly used in high-value amenity areas.
4. **Mixed stands and resistant species:** Establishing mixed-species stands and incorporating resistant species, such as specific hardwoods, are commonly used control strategies.
5. **Phytosanitary controls:** Preventing the intercontinental and interregional spread of disease is essential. This can be achieved by restricting shipments of logs and unseasoned wood that may harbor incipient decay.

Chapter Two:

Study area: Hafir forest

II.1. Geographical Location

The forest territories of Tlemcen, Maghnia, and Tlemcen National Park are home to this woodland, which spans 1653 hectares and falls under the administrative jurisdiction of the communes of Ain Ghoraba and Sabra. The Hafir Forest is situated between latitudes 34°77'79.4"N and 34°77'70.3"N, and longitudes 0°14'29.45"W and 0°14'29.44"W (Tab. 3). It lies southwest of Tlemcen, at an altitude ranging from 1000 to 1420 meters. Estimates of its total surface area vary between 9872 hectares and 10,157 hectares (Bendjebbar *et al.,* 2023) (Fig. 8).

Table 03. Geographic coordinates of the Hafir forest (Bouhraoua, 2003).

Forest	Geographic coordinates	Distance to the sea	Military survey map
Hafir	Latitude: X1 = 105.2 km X2= 127.1 km Longitude: Y1 = 163.6 km Y2 = 178.3 km	60	Terny (1922 type-sheet no◦ 299 & 300

This forest geographically belongs to the commune of Terny, it is bounded by:

- To the North: the village of El Guenaine and the commune of Sebra
- To the Northwest: Zelboun and Ain Douz
- To the Northeast: the forest of Zariffet
- To the East: the national road leading to Tlemcen and the commune of Beni Mester
- To the West: the commune of Beni Bahdel
- To the South: the village of Beni Bahdel, the road leading to Beni Snous, the commune of Ain Ghoraba, and Terny
- To the Southwest:the forest of Moutas

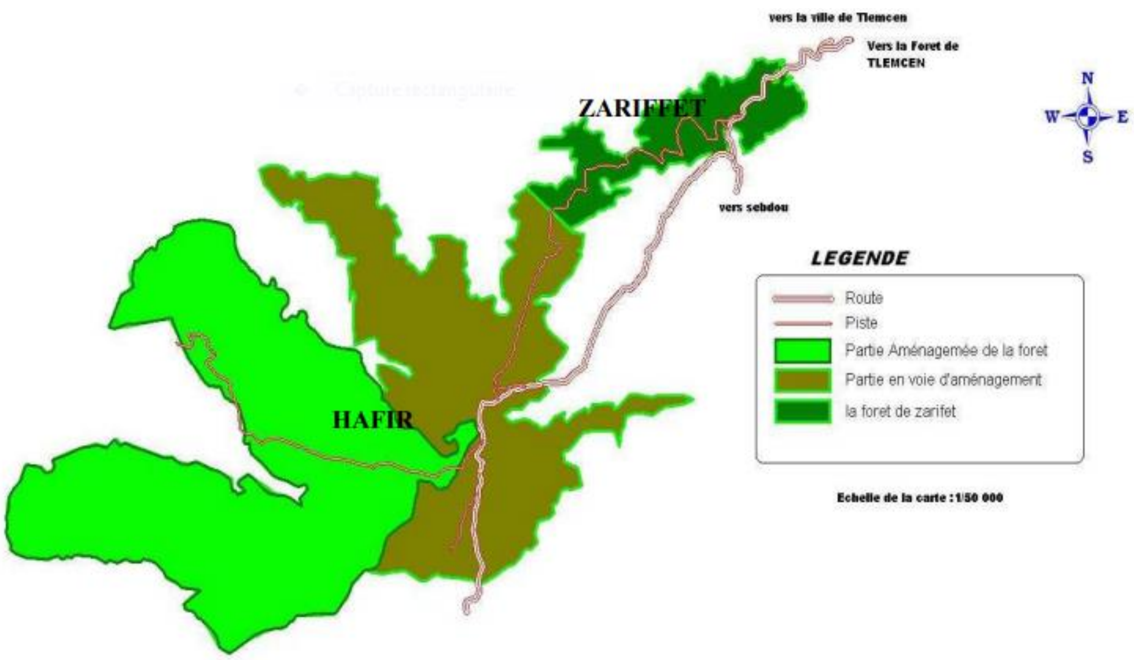

Figure 08. Location of the Hafir-Zariffet forest massif (Forest Conservation of Tlemcen, 2007)

II.2. Climate and Hydrology

The region is characterized by a sub-humid Mediterranean climate, with cool winters and dry summers. Precipitation is highly irregular, with alternating periods of heavy rainfall and drought, leading to an inconsistent water regime. Annual rainfall ranges between 650 mm and 1,000 mm, although some sources report values between 500 mm and 600 mm. Groundwater represents an important source of drinking water in the area (Benhachem & Harrache, 2020).

The climate is characterized by a four-month summer drought starting in June, during which only about 7% of the annual precipitation occurs. The average annual rainfall is 658 mm, and the mean annual temperature is 13.2°C. The hottest month reaches an average maximum temperature of 29.1°C, while the coldest month records an average minimum of 2.4°C. According to the Emberger pluviometric quotient ($Q2 = 80.10$), the climate is classified as moderately humid with cool winters, based on the reference climatic period (1975–2007) (Benabdallah & Amrani, 2014).

II.3. Hydrography

The "Oued Tafna," the most significant river in the region of tlemcen, crosses the Hafir area. The “Oued Boumeroune” a single river that flows from east to west and corredponds to the Tafna basin, flows through the southern portion of the Hafir region (Elmi, 1970).

II.4. Geology and Pedology

The Hafir forest rests on a massif dating from the late Jurassic, composed mainly of Séquanian sandstone and Quaternary alluvium (Letreuch, 2009).

The stratigraphic data of Tlemcen Mountains have been extensively described in the work of Kaid Slimane (1999), we briefly cite:

- The Boumediene sandstones or Lutasicas sandstones, represented by sediments essentially terrignous with molassic affinities
- The dolomites of Terny (Lower Tectonics).

The forest soil originates from limestone bedrock and is classified as fersiallitic brown soil. Although it is thick and porous, it has a low water retention capacity. However, it is rich in essential base cations such as potassium (K^+),magnesium (Mg^{2+}), and calcium (Ca^{2+}). This soil type supports typical Mediterranean vegetation, particularly holm oak (*Quercus ilex*) and Aleppo pine (*Pinus halepensis*).

Additionally, the Hafir Forest is impacted by atmospheric pollution due to its proximity to major roads with a high traffic volume (approximately 500 vehicles per day). This location makes the forest a key site for studying the effects of pollution on ecosystems (Benhachem & Harrache, 2021).

II.5. Vegetation and Biodiversity

The Hafir Forest is a heterogeneous ecosystem characterized by the presence of three oak species: *Quercus suber* (cork oak), *Quercus ilex* subsp. *ballota*, and *Quercus faginea* subsp. *broteroi*. Other prominent tree species include the wild olive (*Olea europaea* L. subsp. *europaea*), narrow-leaved ash (*Fraxinus angustifolia* subsp. *oxycarpa*), prickly cedar (*Tetraclinis articulata*), and oxycedar juniper (*Juniperus oxycedrus*).In lower altitude areas, species such as Aleppo pine (*Pinus halepensis*), stone pine (*Pinus pinea*), common cypress (*Cupressus sempervirens*), and eucalyptus have been introduced through reforestation programs (Maazaouz, 2022).

The shrub layer includes *Arbutus unedo* (strawberry tree), *Erica arborea*, *Phillyrea angustifolia*, *Crataegus oxyacantha* (hawthorn), *Juniperus oxycedrus*, *Rosa canina*, *Genista tricuspidata*, *Calycotome spinosa*, *Cistus monspeliensis*, and *Chamaerops humilis*(Kouider *et al.,* 2019).

The Tlemcen Mountains, including the Hafir Forest, serve as an important model for understanding the evolution of flora and vegetation. The diversity of landscapes and biological conditions in this region has led to the development of mixed stands of holm oak (*Quercus ilex*) and Zeen oak (*Quercus canariensis*), particularly in the Hafir and Zarifet woodlands. In contrast, other areas exhibit more degraded forest formations, primarily due to human activities and environmental pressures (Taibi, 2016; Bouazza *et al.,* 2020).

In the Mediterranean basin, the forest area requires some ecological and socio-economic importance. In addition, Algerian forests, like Mediterranean forests, present significant natural resources, including a proven floristic diversity (Quézel & Médail, 2003).

The Tlemcen Mountains forests offer a very interesting model for studying the flora and vegetation evolution. The variety of landscapes, but also their differences, remain very remarkable, their distribution is conditioned by a large number of ecological factors. They are characterized by mixed groups of holm oak and Zeen oak in Hafir and Zarifet forests. Elsewhere, these are degraded groupings (Dahmani, 1997).

II. 5.1. The holm oak (*Quercus ilex*)

❖ History of Holm Oak

The holm oak (*Quercus ilex*) has been present in the Mediterranean Basin since at least the Miocene epoch. Its distribution progressively expanded from southern to northern regions. In central and southern Spain, it was particularly abundant as early as 9500 B.P. (Before Present), while prior to that time, it likely persisted in localized warm refugia. After 5000 B.P., similar proportions of *Q. ilex* and the deciduous *Q. humilis* (also referred to as *Q. pubescens*) were recorded in northeastern Spain.

In the northwestern Mediterranean Basin, the expansion of *Q. ilex* appears relatively recent, progressively replacing forests dominated by *Q. humilis*. For instance, in Lower Provence (southern France), *Q. ilex* became dominant only after the Subatlantic period (circa 2000 B.P.), a development that coincided with intensified human activity and agricultural expansion during the Gallo-Roman period. A comparable pattern was observed in the Eastern Mediterranean Basin, where an increase in *Q. ilex* pollen is associated with anthropogenic pressures at the end of the Atlantic period (circa 4700 B.P.).

In North Africa, extensive stands of holm oak likely existed long before these dates, but a marked increase in its distribution, likely tied to human influence, is also documented (Rodà *et al.,* 1999).

❖ Ecological and geographic distribution

Holm oak (*Quercus ilex* L.) is one of the 640 documented species listed in the *Plants of the World Online* database. It ranks among the most significant clades of woody angiosperms in the Northern Hemisphere in terms of species diversity, ecological dominance, and economic importance. The genus *Quercus* holds a central role both ecologically and economically, encompassing both deciduous and evergreen taxa. In the Mediterranean Basin, holm oak stands out as a key sclerophyllous evergreen tree species, widely distributed across the Iberian Peninsula, southeastern France, Morocco, Algeria, and Italy (Fig. 09). This species is classified into two subspecies: *Quercus ilex* subsp. *ilex* L. and *Quercus ilex* subsp. *Ballota* (Desf.) Samp. (Dolores Rey *et al.,* 2022) (Fig. 10).

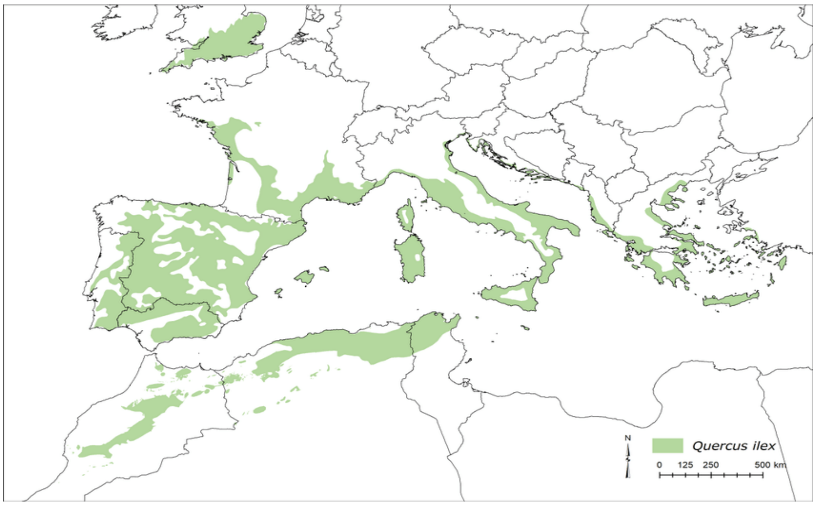

Figure 09. Chorological map of holm oak (*Quercus ilex*) (Pausas & Millán, 2019).

Figure 10. Field image of *Quercus ilex* (Holm Oak) in its Natural Habitat Hafir Forest (Original)

Holm oak thrives in Mediterranean climates characterized by summer droughts and cold to mild, humid winters. It grows naturally across a wide altitudinal gradient, from sea level up to 2,500 meters above sea level (m.a.s.l.), due to its remarkable ability to cope with drought and thermal stress through efficient stomatal regulation and photoprotective mechanisms (Ballesteros *et al.,* 2023).

❖ . Biology of Holm Oak

❖ Taxonomy and Chemical Characteristics

Holm oak (*Quercus ilex* L.) is a monoecious, wind-pollinated tree classified within the subgenus *Sclero phyllodrys* O. Schwarz. It can reach up to 27 meters in height, although it rarely exceeds 15 meters under natural conditions (Rodà *et al.,* 1999). Two main subspecies are recognized:

- *Quercus ilex* subsp. *ilex*, primarily found in coastal areas.

- *Quercus ilex* subsp. *ballota* (Desf.) Sampaio (syn. *Q. ilex* subsp. *rotundifolia*), predominant in interior landscapes.

The species is characterized by a broad canopy of ascending branches and a relatively short trunk, which can occasionally reach up to 2 meters in diameter. As one of the most widespread tree species in Portugal and Spain, *Q. ilex* and its subspecies have long been valued for their timber. Furthermore, their acorns have historically served as an important staple food.

The bark of *Quercus ilex* is primarily composed of suberin (ω-hydroxy acids), polysaccharides, lignin, and various extractives. It also contains catechins and several phenolic acids, including 4-hydroxybenzoic, caffeic, coumaric, ferulic, and gentisic acids (Eva Sánchez-Hernández *et al.,* 2022).

❖ Leaf morphology and photosynthetic adaptations

Holm oak (*Quercus ilex*) develops dense, evergreen canopies with small, leathery (coriaceous) leaves. The upper surface of the leaf is dark green, while the underside is covered with a dense layer of white hairs, which helps retain moisture and limit transpiration (Fig. 11). The stomata, measuring 22–27 µm in length, are restricted to the abaxial (lower) surface and are protected beneath stellate hairs, with a density ranging from 100 to 500 stomata/mm². This specialized structure ensures highly efficient water regulation. Furthermore, the thick upper epidermis and cuticle act as protective barriers against excessive water loss and high temperatures (Rodà *et al.,* 1999).

As a shade-tolerant species, holm oak plays a dominant role in late-successional forest stages. Its high chlorophyll content allows for year-round photosynthesis, even under low-light conditions. The Leaf Area Index (LAI) can reach 4.5 m², optimizing light capture. Interestingly, LAI remains relatively stable regardless of water availability, and in dense forests, foliage is mainly concentrated in the uppermost meter of the canopy.

Figure 11. Morphologizl Detail of *Quercus ilex* Leaf – Hafir Forest, Tlemcen (Original)

❖ Wood Structure and Hydraulic Properties

The wood of holm oak exhibits a dense, diffuse semi-porous structure, with vessel diameters ranging from 50 to 150 micrometers. The vessels are irregularly distributed and gradually become narrower toward the outermost growth rings. Compared to ring-porous species like deciduous oaks, the vessels are relatively short and narrow. The identification of annual growth rings is often challenging due to the presence of false rings and the obscuring effect of prominent multiseriate medullary rays. As a result, available data on radial growth rates remain limited.

Thanks to its hardness and high density, holm oak wood is traditionally used for making carts, tool handles, hand planes, various carpentry tools, gear teeth, and parquet flooring. Its specific vessel anatomy results in slow sap flow velocities during midday peaks, ranging from 1 to 6 meters per hour (equivalent to 0.3–1.7 mm/s). In this type of wood structure, cavitation events tend to cause considerably less damage than in ring-porous species (Rodà *et al.,* 1999).

❖ Root System and Drought Adaptations

Holm oak develops an extensive root system that is essential for its survival in arid environments. In its first year, a seedling can produce a taproot reaching depths of 60 to 80 cm, allowing it to access deep soil moisture. As the tree matures, it forms a complex network of lateral roots that can extend several meters from the trunk. In coppiced individuals, the root crown becomes particularly thick and massive due to repeated resprouting, which leads to the progressive accumulation of significant root biomass over time.

❖ Threats and Decline

Currently, *Quercus ilex* is increasingly threatened by a combination of anthropogenic and environmental pressures. These include an aging tree population, overexploitation coupled with poor natural regeneration, mismanagement of grazing practices, as well as biotic stresses (e.g., *Phytophthora cinnamomi*) and abiotic challenges such as extreme temperatures and prolonged droughts. Together, these factors are believed to drive the so-called *holm oak decline syndrome*, characterized by rising mortality rates and a marked degradation of the dehesa ecosystem (María-Dolores Rey *et al.,* 2022).

In transitional zones between different climatic regimes, the distribution of *Quercus ilex* is often constrained by intense competition with other species, particularly congeneric oaks. This interspecific competition for key resources such as water, nutrients, and light tends to favor species that are better adapted to the prevailing environmental conditions of these ecotones, thereby limiting the expansion and dominance of *Q. ilex*.

Over recent decades, tree mortality and forest decline have increased globally, with rising incidence often linked to climate change. These decline dynamics carry significant ecological implications, particularly as they affect the capacity of terrestrial ecosystems to function as carbon sinks. Although the primary physiological drivers of tree mortality are related to water and temperature stress, climatic extremes can also amplify the occurrence and intensity of other disturbance factors, including wildfires and pathogenic outbreaks.

II.5.2. The cork oak (*Quercus suber* L.)

The cork oak (*Quercus suber* L.), an evergreen tree belonging to the Fagaceae family**,** comprises 531 species worldwide, with oaks widely represented in the Northern Hemisphere. Among them, the cork oak (*Quercus suber* L.) is a strictly Mediterranean species distributed in the western Mediterranean Basin, spanning southwestern Europe and North Africa an ecologically and socioeconomically sensitive region (Bendjebbar *et al.,* 2023) (Fig. 12).Cork oak woodlands play a fundamental role in biodiversity conservation by providing habitats for various animal species, including endangered ones. These forests contribute significantly to soil conservation by preventing erosion, improving water retention, and regulating the hydrological cycle. They also play a crucial role in carbon sequestration, retaining approximately 14 million tons of CO_2 annually, which underscores their importance in mitigating climate change (Rego *et al.,* 2023).

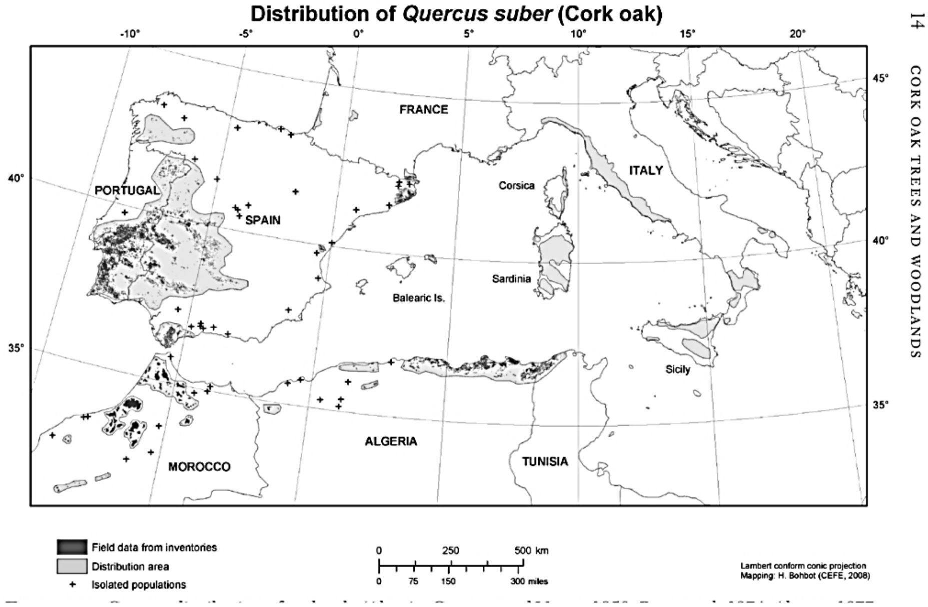

Figure 12. Current distribustion of Cork Oak. (Algeria, Italy, Morocco, Spain, Tunisia) (Pausas *et al.,* 2009).

II.5.3. The Zean oak (*Quercus canariensis*).

The zeen oak (*Quercus canariensis* willd.) is a deciduous to semi-evergreen oak species endemic to North Africa, particularly Algeria, Morocco, and Tunisia, as well as southm Europe, including spain and Portugal (Quézel & Médial, 2003). In Algeria, it is mainly found in humid and sub-humid bioclimatic zones, especially in the Kabylie mountains and the Tlemcen region, including the Hafir Forest (Benabadji *et al.,* 2007). Zeen oak may grow to 30 meters in height and likes deep, fertile, and moist soils, with less drought tolerance that Holm oak (*Quercus ilex*). Its broad, thin leaves are somewhat serrated, and it produces huge acorns that mature over two years.

In Algeria, the zeen oak holds an important place in the economic hierarchy of the forest species: 65, OOO ha. Concentrated in the North, it colonizes today some coastal chains of the tell Atlas where it seems to find conditions favorable for its development. The factors influencing the evolution of this species are the climate (cool and well watered stations) and the altitude (the zeen oak thrives especially in humid bioclimatic zones) (Zerizer & Mansseri, 2003).

II.5.4. The olive tree (*Olea europaea)*

According to Crisosto *et al.* (2011), the olive tree belongs to the Oleaceae family (*Olea europaea*). This species is part of the order Lamiales and includes six recognized subspecies: *zuropaea, maroccana, cerasiformis, laperrinei, gaunchica, and cuspidate* which together from the O. europaea complex. Whithin the subspecies europaea, two main varieties are distinguished:

- ***Var.sylvestris***, which includes the wild forms of the olive tree
- ***Var. europaea***, which includes the cultivated forms (Green, 2002; Vargas *et al*., 2000)

These individuals have an allogamous mode of reproduction and some of them are self-incompatible or male-sterile (Besnard *et al.,* 2000; Breton *et al.,* 2017).

At low altitude appears the prickly cedar (*Tetraclinisarticulata (Vahl*) *Mast*.) and oxycedar juniper (*Juniperus oxycedrus L. subsp. Oxycedrus*) (Mostefia, 2011). The Aleppo pine (Pinus halepensis Mill.), the pinion pine (*Pinus pinea L.*), the common cypress (*Cupressus sempervirens L.*), and eucalyptus meet in certain degraded cantons, where they were introduced during the last reforestation programs (Bouharoua, 2003).

Among the most common understory species, we cite *Hedera helix L. subsp. Helix, Loniceraimplexa Aiton, Smilax aspera L., Rubus ulmifolius schott , Daphne gnidium L, Arbutus unedo L., Ruscusaculeatus L., Erica arborea L., Rosmarinus officinalis L. and Pteridum aquilinum (L.) Kuhn subsp. Aquiline*. In the degraded, warmer areas, wz can find more secondary species such as *Quercus coccifera L. and Juniperus oxycedrus L. subsp. Oxycedrus, and also cistus (Cistus albidus L., Cistus ladanifer L., Cistus monspeliensis L., Cistus salviifolius L. and Cistus certicus L.,), Ampelodesmps mauritanicus (Poir.) T. Durand & Schinz and Chamaerops humilis L.* (Haffaf, 2011).

Chapter Three: Materials and Methods

III.1. Introduction

The Hafir Forest represents a significant natural ecosystem, characterized by its rich biodiversity and ecological importance. Located in the Tlemcen region, this forest is home to a heterogeneous vegetation stand composed mainly of unique plant species.

However, in recent years this ecosystem has faced significant environmental challenges, particularly the spread of cryptogamic diseases, which pose a serious threat to its survival. Among the primary causes of decline, fungal endophytes are particularly concerning, as they can persist in plant tissues for extended periods without manifesting symptoms.

Our study, conducted in the Hafir Forest, aims to analyze and understand the factors contributing to tree decline, with a focus on Holm Oak (*Quercus ilex*) and Cork Oak (*Quercus suber*). These species were selected due to their ecological importance, their dominance in the region, and their vulnerability to fungal pathogens. The research involves isolating, identifying, and characterizing phytopathogenic fungi affecting these trees, particularly those exhibiting symptoms such as leaf discoloration, needle and leaf loss, wilting, and progressive branch dieback.

In order to realize this objective, the study is partitioned into two principal phases:

1. Fieldwork, which encompasses site selection, tree sampling, and documentation of symptoms.

2. Laboratory analysis, wherein the collected samples will be scrutinized to identify fungal species correlated with tree decline.

III.2. Characteristics of the study site and sampled trees

To better understand the presence of fungal species and their potential role in forest decline, a study was conducted in the Hafir Forest, located in the Tlemcen region of northwestern Algeria. This site was selected due to visible symptoms of decline affecting several native tree species, particularly oaks. Field surveys were carried out to characterize the site in terms of its geographical and topographical features, including elevation, slope orientation, and general ecological context. This characterization provides a basis for interpreting the interactions between environmental conditions and fungal colonization

III.2.1. Geographical and topographical survey

The geographical and topographical data related to the selected study site were compiled and are presented in the table 6. These observations include key parameters such as location, altitude, slope, and exposure, which are essential for understanding the ecological context of the Hafir Forest.

Table 06. Main geographial and topographical characteristics of the Hafir Forest (Tlemcen)

Wilaya	Tlemcen
Forest	Hafir forest
Sector/ Canton	Tlemcen National Park, Central Zone
Geographical Coordinates	34°51′00″ N – 01°19′00″ W
Topography	Mountainous area
Slope Exposure	North-Northeast
Average Altitude (m)	1100 m
Slope Degree	Moderate steep
Distance from the Sea	70km (straight line to Mediterranean coast)

The Hafir Forest is situated within Tlemcen National Park in northwestern Algeria. Geographically, it lies approximately 35 km southeast of the city of Tlemcen, at coordinates 34°46′37″ N, 1°29′39″ W.

The terrain of the Hafir Forest is predominantly mountainous, with elevations ranging from 900 meters along access routes to peaks reaching 1140 meters. The area features moderate to steep slopes, with predominant slope exposure facing north to northeast.

III.3. Prospecting and sampling

As part of this study, the Hafir Forest was selected as the research site due to its ecological importance within Tlemcen National Park. Situated in northwestern Algeria, in the wilaya of Tlemcen, within the municipality of Terni, this forest covers an area of 9,872 hectares (Bendjebbar *et al.,* 2023). It represents a vital component of the regional forest ecosystem, contributing significantly to biodiversity conservation and the maintenance of ecological balance.

III.3.1. Characteristics of the study station and sample trees

III.3.1.1. Choice of the station

Hafir Forest, a key component of Tlemcen National Park in northwestern Algeria, was selected as the study site due to its documented history of forest decline, particularly associated with cryptogamic diseases. The forest has been significantly affected by fungal pathogens, which have contributed to severe dieback and mortality, especially among holm oak (*Quercus ilex*) and cork oak (*Quercus suber*) populations. Previous studies have identified pathogenic fungi, including *Biscognauxia mediterranea* and *Diplodia sp.*, as major contributors to forest decline in Mediterranean ecosystems, causing cankers on trunks and branches, crown dieback, and overall tree weakening.

The site is characterized by a combination of natural and planted stands, with afforestation efforts dating back to the 1960s. However, in recent decades, Hafir Forest has shown increasing signs of degradation, including widespread defoliation, necrotic lesions on branches and trunks, and sudden tree mortality, symptoms typically associated with fungal infections. The presence of symptomatic and dead trees, particularly in areas with poor drainage and compacted soils, suggests a high susceptibility to fungal pathogens, exacerbated by climatic stressors such as drought and extreme temperatures. The selection of Hafir Forest as a research site enables a detailed investigation into the role of cryptogamic diseases in Mediterranean forest decline. By assessing the extent of fungal infections and their ecological impact, this study aims to enhance understanding of disease dynamics in oak woodlands and to support future conservation and forest management strategies.

III.3.1.2. Choice of trees

Holm oak (*Quercus ilex*) and cork oak (*Quercus suber*) were chosen as the focus of this study due to their ecological, economic, and environmental significance in Mediterranean forest ecosystems, particularly in Hafir Forest. These two species are among the most dominant and long-lived evergreen oaks in the region, playing a crucial role in maintaining biodiversity, stabilizing soils, and regulating local climate conditions.

Both species are highly resilient to Mediterranean climatic conditions, exhibiting some tolerance to drought and adaptability to poor soils. However, in recent years, they have shown alarming signs of decline, primarily due to abiotic or biotic stressors, such as primary or secondary fungal attacks. These pathogens weaken the trees, making them more vulnerable to secondary infections and environmental stressors, such as prolonged droughts and rising temperatures.

Additionally, holm oak and cork oak hold significant economic and cultural value. Cork oak is particularly important for cork production, supporting a sustainable industry that benefits local economies. Holm oak, on the other hand, plays a vital role in traditional agroforestry systems, such as the ***"Dehesa"*** in Spain and ***"Montado"*** in Portugal, where it provides acorns for livestock and supports diverse flora and fauna. The decline of these species not only threatens forest health but also disrupts livelihoods and ecosystem services.

Given their ecological importance and increasing vulnerability to forest decline, the study of these two species in Hafir Forest is essential to understand the impact of cryptogamic diseases and their biology.

III.3.1.3. Dendrometric measurements

Dendrometric measurements were carried out to evaluate the size and overall condition of the trees in the Hafir Forest. Specifically, the following two parameters were recorded for each tree:

- **Tree Height**: The total height of each tree was measured using a Blum-Leiss device, which provides accurate readings from the base to the top of the tree.
- **Trunk Circumference**: The circumference of the tree trunk was measured at 1.3 meters above the ground (breast height) using a measuring tape. This standard measurement is essential for assessing the tree's size and health.

III.3.1.4. Health reports

The evaluation of tree vitality and stand health was based on a combined analysis of canopy condition and trunk integrity. The methodology primarily involved a systematic assessment of foliar deficit and visible trunk abnormalities.

Crown condition is a key indicator of tree vigor and was assessed using two main symptoms: the degree of defoliation, which reflects foliage loss, and discoloration, which indicates abnormal changes in leaf pigmentation. Defoliation was classified into five distinct categories, each corresponding to a specific health status, allowing for a structured diagnosis of tree decline (Belkhir *et al.,* 2011; Zerrouki *et al.,* 2022) (Tab. 04, Tab. 05).

In parallel, a detailed inspection of the trunk was carried out to detect fungal infections, cankers, lesions, cracks, or structural anomalies that could compromise tree stability and function. Assessments of bark condition and decay provided essential insights into potential cryptogamic diseases (DSF, 2018).

Table 04.Tree decline evaluation scale (Zerrouki *et al.,* 2022).

Class	Foliage loss (%)	Tree health status
1	<25%	Light
2	> 30% ≤ 60%	Moderate
3	> 65% ≤ 95%	Severe
4	100%	Very Severe

Table 05.Leaf discoloration classes (Zerrouki *et al.,* 2022)

Class	Proportion of discolored foliage	Class meaning	Type of coloration
1	0-10%	No discoloration	Normal coloration
2	> 10% ≤ 25%	Slight discoloration	Abnormal coloration
3	> 25%≤ 60%	Moderate discoloratio	Abnormal coloration
4	>60%	Severe discoloration	Abnormal coloration

By systematically evaluating these indicators, the methodology offers a reliable framework for detecting early signs of stress, whether caused by biotic agents such as fungal pathogens, or abiotic pressures like drought. This approach is essential for developing targeted management strategies aimed at preserving forest ecosystems in the face of increasing environmental stressors.

III.3.2. Sampling Method

For this study, a total of 30 samples were collected from symptomatic trees in the Hafir Forest, with a focus on Holm oak (*Quercus ilex*) and Cork oak (*Quercus suber*), along with a few additional samples from Aleppo pine (*Pinus halepensis*) and Cade juniper (*Juniperus oxycedrus*).

Sampling was conducted based on visible symptoms of fungal infection. Trees exhibiting signs of cryptogamic diseases, such as defoliation, discoloration, or branch dieback, were selected. To ensure a diverse representation of affected trees, a systematic approach was employed: the first symptomatic tree was numbered, and subsequent trees were selected using the "nearest neighbor" method, regardless of their size or overall health condition.

For each sample, the focus was placed on the aerial parts of the trees, specifically collecting portions of branches, stems, and xylem near the collar. These parts were carefully excised to target tissues most likely to be affected by fungal infections. After collection, each sample was placed in a sterile plastic bag labeled with essential reference information, including the sample number, host species, forest name, study site, and sampling date. All samples were then transported to the laboratory and stored at 5°C in a refrigerator until further analysis.

III.4. Isolation, identification, and characterization of the isolated fungi

III.4.1. Cultural media used

The selection of an appropriate culture medium is critical for promoting optimal fungal growth and accurately identifying phytopathogenic fungi. In this study, three types of culture media were used for the isolation and purification of fungal strains: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Agar-Agar (AA).

- ✓ **Potato Dextrose Agar (PDA):**

PDA **is** one of the most commonly used media for cultivating a wide range of fungal species. It provides a rich source of nutrients derived from potato infusion and dextrose, supporting rapid mycelial growth and sporulation. Its balanced composition makes it particularly suitable for general isolation and the maintenance of fungal cultures.

- ✓ **Malt Extract Agar (MEA):**

MEAas noted by Viegas *et al.* (2021), is a nutrient-rich medium widely used for the cultivation of environmental and fastidious fungi. Due to its high concentration of maltose and other saccharides, it serves as an excellent energy source, especially for the growth of yeasts and molds. The presence of malt extract supports the development of a broad range of fungal species, particularly those associated with wood decay and phytopathogenic activity.

- ✓ **Agar-agar (AA):**

Agar-Agar (AA), composed primarily of purified agar, was used especially for fungal strains with poor sporulation on nutrient-rich media. Due to its low nutrient content, this mediumlimit excessive vegetative growth and instead promotes the development of reproductive structures, which are essential for the morphological identification of certain fungal species.

III.4.2. Sample Preparation

Fungal isolation was performed by cutting leaves and branches into small fragments, approximately 0.5 × 0.5 cm in size. These fragments were then immersed in a 1% sodium hypochlorite solution for one minute to achieve surface sterilization. After disinfection, the samples were rinsed three times over a 30-minute period with sterile distilled water to eliminate any residual chemicals. Finally, the fragments were dried using sterile filter paper prior to the isolation process (Fig.13).

Figure 13. Prepared and disinfected samples prior to isolation stage (Original)

III.4.3. Fungal Isolation

Using a scalpel regularly sterilized with 70% ethanol, a small fragment (0.5 × 0.5 cm) of each sample was cut and placed onto 90 mm diameter Petri dishes containing the prepared culture media (PDA, MEA, or Agar-Agar). The fragments were carefully positioned to ensure good contact with the medium.

The Petri dishes were then incubated in the dark at 25°C for a period of 5 to 10 days. During incubation, fungal growth emerging from the plant tissues was monitored daily. Pure cultures obtained from this process were later used for morphological observations and further identification procedures (Fig. 14).

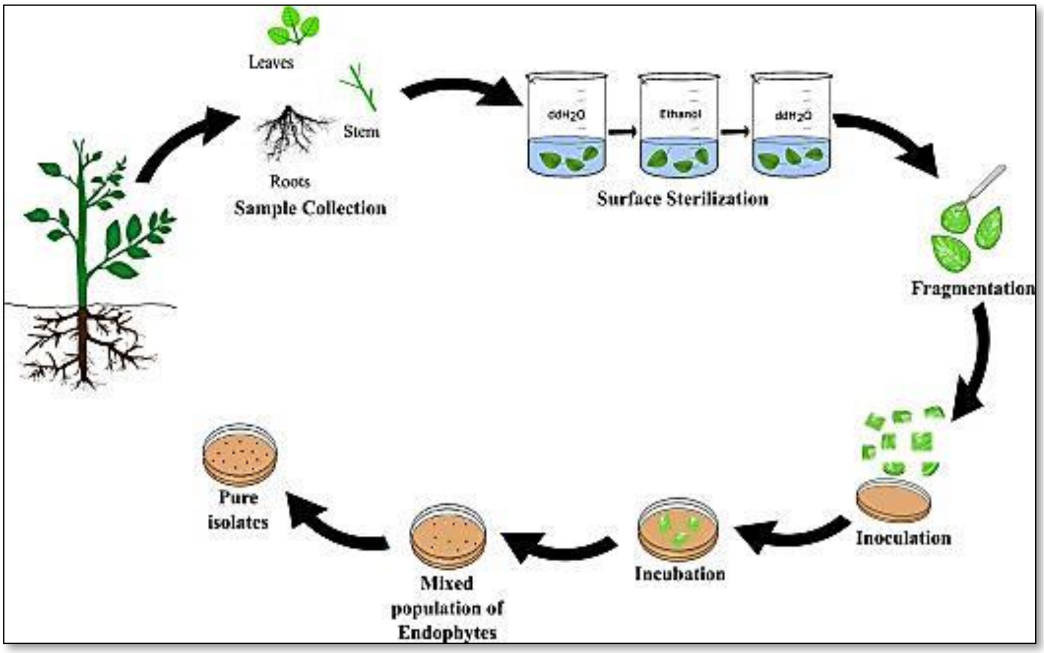

Figure14. Stepwise procedure for endophyte isolation and cultivation (Manias & Soni, 2020).

III.4.4. Purification of fungal colonies

After the development of fungal colonies, small fragments were carefully transferred using sterile Pasteur pipettes onto fresh Petri dishes containing the same type of medium. This subculturing step aimed to obtain pure fungal cultures by isolating individual strains free from contaminants.

The plates were incubated in complete darkness at 23°C for 3 to 5 days. This process was repeated as needed until pure fungal strains were successfully obtained (Fig. 15).

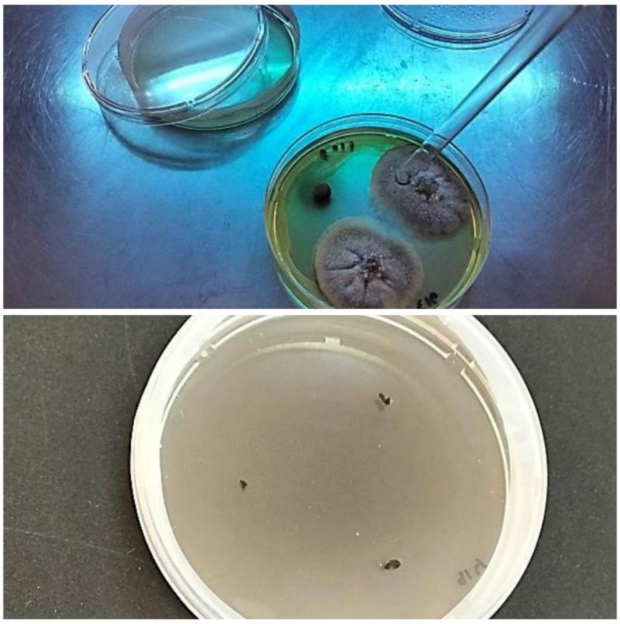

Figure 15. Purification process of fungal isolates on PDA medium (Original)

III.4.5. Macroscopic and microscopic identification of isolated strains

Fungal species identification was based on the observation of both macroscopic and microscopic morphological characteristics.

a. **Macroscopic characterization** was carried out on solid culture media by evaluating the colony appearance on both the upper and reverse sides. Pure fungal isolates were grouped and categorized according to their macroscopic features in order to select representative strains from each group for detailed identification. The macroscopic traits assessed included colony texture, size, surface relief, color (obverse and reverse), odor, and margin shape. All characteristics were examined visually on Petri dishes under ambient light conditions.

b. **Microscopic analysis** was performed using a compound light microscope equipped with imaging software and a digital camera. For slide preparation, a small piece of mycelium was carefully taken from the margin of the thallus using a sterile needle and placed on a clean glass slide containing a drop of lactic acid to maintain cellular isotonicity. A coverslip was gently applied to the preparation, followed by a drop of immersion oil.

Microscopic observations were conducted at magnifications of ×10, ×40, and ×100. Fungal species were identified based on key morphological criteria such as vesicle shape, the arrangement and morphology of spores and conidia, septation of conidia, and hyphal compartmentalization.

Fungal identification was supported by comparisons with established identification manuals and taxonomic keys, including Barnett and Hunter (1972), Botton *et al.* (1990), Champion (1997), and Plaat-Niterink (1981).

III.4.6. Statistical analysis

a. The **Shannon diversity index** (H') was calculated to assess the diversity of fungal species isolated from leaf and branch samples. This index accounts for both the number of species (species richness) and their relative frequency. The calculation was based on the formula:

$$\mathbf{H'=-\sum (\mathit{Pi} \cdot \ln \mathit{Pi})}$$

where *Pi* represents the proportion of each species relative to the total number of isolates. Data from positive samples were used, and the index was estimated both globally and for each host tree species, in order to compare fungal diversity across different forest tree species.

b. Fisher's test

To assess the potential association between the host tree species and the presence of isolated fungi, a **Fisher's exact test** was performed for each fungal species. The data were organized into 2×2 contingency tables showing the frequency of presence and absence of each fungus according to the main forest tree species sampled. The test was carried out using the **R software 2019**. This test is particularly suitable for small sample sizes and allows the determination of whether the distribution of a fungus differs significantly depending on the host tree species. The resulting p-values and odds ratios helped identify statistically significant associations between certain fungi and their plant hosts.

III.4.7. Antagonism Test

The antagonism test is a biological method used to evaluate the ability of certain microorganisms to inhibit or suppress the growth of pathogens.

According to Hibbat *et al.,* 2005:The antagonistic activity test was conducted using a dual culture technique on a PDA medium. This method involves two mycelial plugs (0.5 cm in diameter) were excised from the edges of actively growing cultures of the two fungi included in the test: the pathogenic species and the potential antagonist.

Two plugs, one of *Trichoderma sp.* and the other of *Diplodia sp.*, were placed in the same Petri dish (90 mm in diameter) previously filled with PDA medium, along a diametral axis at 3 cm and equidistant from the center of the dish. The plates were incubated at 20°C in complete darkness for five days. Three (3) replicates were performed for each fungal combination (Fig. 16).

Mycelial growth of both fungi was evaluated daily. The radial growth of the pathogenic fungus, specifically on the side facing the antagonist, was measured directly across the diameter of the Petri dish. These measurements were taken every 24 hours over a 5-day period at the same time each day to ensure consistency.

Figure16. Assessment of fungal antagonistic in culture (Original).

Chapter Four

Results and Discussion

1. Dendrometric Survey

A total of 30 symptomatic trees were examined during the study, including 14 holm oaks (*Quercus ilex*), 10 cork oaks (*Quercus suber*), 5 Cade juniper (*Juniperus oxycedrus*), and 1 Aleppo pine (*Pinus halepensis*). Key dendrometric parameters were recorded, namely trunk circumference and total tree height. These measurements were then classified into categories based on size ranges.

The total height of the sampled trees ranged from 2 to 8 meters, while other mature trees within the Hafir Forest can reach up to 15 meters. This indicates that the observed stand corresponds to the sapling and early pole stage (gaulis) in silvicultural development. The sampled trees fall into classes 1, 2, and 3, highlighting a significant variety in height from small individuals to more developed specimens. This variation reflects differences in growth dynamics and developmental stages within the forest ecosystem.

In the study area of Hafir Forest, the trunk circumference of trees ranged from 20 cm to 70 cm, which is typical of a relatively young forest population. However, a few individuals had circumferences up to 100 cm, indicating the presence of older or more vigorous trees. Approximately 70% of the sampled trees belonged to class 1, reflecting the dominance of younger growth stages in the selected plot.

The variation observed in circumference classes reveals a wide distribution of tree sizes within the Hafir Forest. While some individuals have small trunk girths, others show significantly larger dimensions. This structural heterogeneity can be attributed to a combination of ecological and biological factors, including tree age, growth rate, interspecific competition, and localized microclimatic conditions. Notably, such diversity in size and developmental stages is characteristic of semi-natural Mediterranean forests, particularly those influenced by past reforestation initiatives and natural regeneration. Additionally, this variation may influence the differential susceptibility of trees to cryptogamic diseases, as younger or stressed individuals with reduced vigor are often more prone to fungal infections. Understanding these structural dynamics is therefore essential for assessing forest health and managing disease impacts in the region.

Research conducted in 2017 by Bougaham et al.identified the presence of the pathogenic fungus Biscogniauxia mediterranea in the Hafir Forest, a known agent of charcoal canker associated with cork oak decline. The study found that approximately 80% of the affected trees fell into the second circumference class (70–109 cm) and were within height classes 2 (6–7 m) and 3 (7–10 m). This suggests that mid-sized trees are particularly susceptible to infection. The spread of this pathogen, coupled with environmental stressors such as drought and high temperatures, contributes to the ongoing degradation of the forest ecosystem.

2. Health assessment

The health condition of a forest tree is typically assessed by examining two key indicators: foliage loss (defoliation) and crown discoloration. These symptoms provide essential clues about the tree's overall vitality and help identify potential stress factors or emerging threats. Defoliation reflects a reduction in leaf density, often caused by biotic or abiotic stress, while crown discoloration may indicate physiological imbalance, nutrient deficiency, or early symptoms of cryptogamic infections, such as those caused by pathogenic fungi. Together, these criteria serve as practical tools for monitoring forest health and detecting signs of decline in individual trees (Fig. 18).

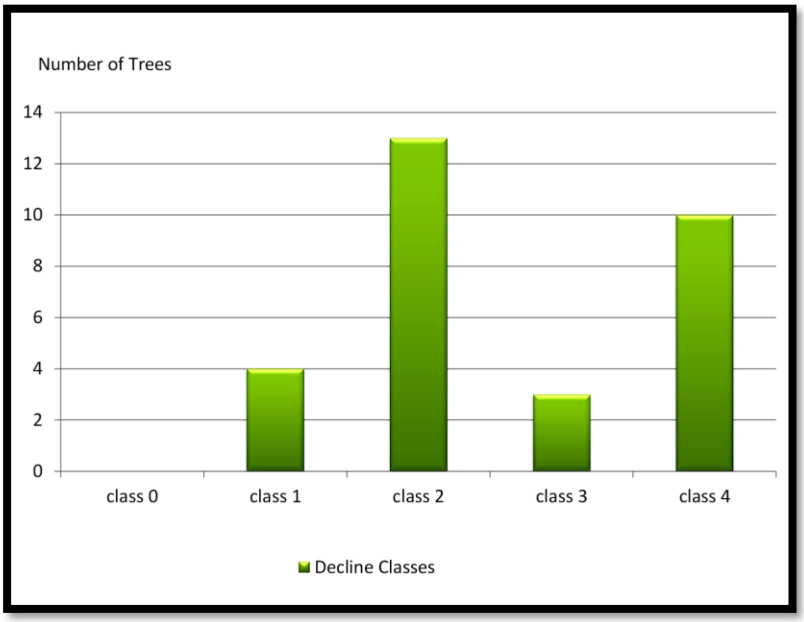

Figure 18. Health status of the sampled trees

In the Hafir Forest, located in the Tlemcen region, a significant decline in tree health has been observed, particularly affecting holm oak (*Quercus ilex*) and cork oak (*Quercus suber*) populations. This decline has caused considerable damage across a wide range of tree species. Analysis of Figure 18 reveals that the deterioration has affected numerous trees, often clustered in groups.

Notably, only trees exhibiting clear signs of decline were included in this study; healthy individualsthose showing no crown discoloration or less than 10% foliage losswere deliberately excluded to ensure that the analysis focused on the most severely affected specimens. This targeted approach allows for a more accurate assessment of the severity and extent of forest decline.

Among the visible symptoms, several key indicators were observed on the branches, including drying and dieback of terminal shoots, the presence of cankers, bark cracking, and in some cases, the appearance of fungal fruiting bodiescharacteristic of certain cryptogamic diseases (Fig. 19).

Figure19. A declining forest stand in Hafir Forest (Original)

A total of 30 trees in Hafir Forest were observed to determine their level of decline using a five-class system ranging from class 0 (healthy) to class 4 (severely declining). The results reveal a concerning trend toward general deterioration, which may be due to environmental stress, pathogenic infections, and other biotic agents.

1. Class 0: healthy trees (0%)

No trees were classified as class 0, indicating a complete absence of symptom-free individuals. This suggests that all observed trees are experiencing some degree of stress or decline, even if mild.

2. Class 1: Sight decline (4 trees; 13, 3 %)

Trees in this category exhibit mild defoliation (10–19%), which may represent early signs of physiological stress. Trees in this class may still maintain moderate resilience, and recovery could be possible if environmental conditions improve. Their presence indicates the early stages of decline, and they may also act as a buffer zone in the forest recovery dynamics.

3. Class 2: Moderate decline (43.3%)

This is the most represented category, with nearly half of the sample (43% of trees) falling into class 2. These trees exhibit moderate canopy loss (20–49%), which is often associated with reduced photosynthetic capacity, decreased vigor, and increased susceptibility to pests and fungal pathogens.

4. Class 3: Advanced decline (10%)

Trees in this class exhibit heavy defoliation (50–89%), structural dieback, and possible necrosis in twigs and branches. These trees are severely affected and likely have compromised vascular systems. At this stage, the chances of recovery are significantly reduced, especially if stressful conditions persist. Such trees often serve as reservoirs for fungal spores and insect pests, thereby contributing to the spread of decline among neighboring individuals.

5. Class 4: sever decline (33.3%)

This class includes the most severely impacted individuals, with over 90% foliage loss, crown dieback, necrotic branches, and often the presence of fungal fructifications or insect boreholes. Notably, all class 4 trees were cork oaks, highlighting the species' extreme vulnerability under the current stress conditions in Hafir Forest.

Diseases caused by pathogenic fungi play a major role in the weakening and death of trees. In Hafir Forest, many trees were found to be completely dried out, corresponding to class 4, the most severe level of decline. Among the trees studied, five had died recently, while others had been dead for a longer time. When a tree is fully dried out, it means it has lost all its internal moisture, and its tissues are too damaged to sustain life. This stage usually occurs after prolonged stress, particularly from fungal infections that target weakened trees and accelerate their decline.

3. Isolation, identification, and characterization of the isolated species

1. Symptomatology

The analysis of samples collected from Hafir Forest, focusing on visible symptoms in species such as Holm oak, Cork oak, Aleppo pine, and Cade juniper, revealed the presence of a wide variety of fungi, both pathogenic and non-pathogenic. A key observation was that all sampled trees, regardless of their age group, exhibited similar types of symptoms. This consistency strongly suggests that the phytopathogenic diseases affecting the forest are mainly caused by obligate fungal pathogens, which rely entirely on living host tissues to survive and spread.

During field surveys in Hafir Forest, different symptoms were observed depending on the affected tree species. One of the most common signs was needle or leaf discoloration, changing from green to yellow or brown, followed by premature leaf drop. The branches also lost their flexibility and turned brown or grey. Other visible signs included reduced tree growth, overall loss of vigor, weakened condition, branch drying, and lack of new shoot development. Although symptoms varied between species, their concurrent presence clearly indicates that the trees are undergoing decline and dieback (Fig. 20).

Figuer 20. Different parts of a tree showing distinct symptoms of decline

The leaves of *Quercus ilex* (Holm oak) in declining trees often exhibit abnormal features compared to healthy individuals of the same species. Under normal conditions, Holm oak leaves are small and evergreen, measuring approximately 3–7 cm in length and 1.5–3.5 cm in width. They have a dark green upper surface and a dense layer of white hairs on the underside, which helps reduce water loss and provides protection against environmental stress. In stressed or diseased trees, however, the leaves may appear smaller, paler, and more brittle, sometimes accompanied by premature leaf drop. These changes indicate physiological stress, often linked to unfavorable environmental conditions or the presence of cryptogamic diseases.

In the Hafir Forest, the leaves of Holm oak show clear signs of foliar discoloration, such as chlorosis (yellowing), necrosis (browning or blackening of tissue), and the presence of necrotic spots or lesions (Fig. 21). In some cases, small black punctiform structures resembling the fruiting bodies of pathogenic fungi are visible, potentially indicating fungal infection. These symptoms suggest a decline in leaf vitality often associated with biotic stress, such as fungal infection.

Figure21. Chlorosis in Holm Oak (*Quercus ilex*) attributed to cryptogamic disease observed in Hafir forest

Also, we noticed the presence of cork oak (*Quercus suber*) showing signs of decline in Hafir Forest, including canopy thinning, leaf yellowing, branch dieback, and bark cracks (Fig. 22). In addition, signs such as blackened, charcoal-like lesions on the bark and necrotic wood lesions were observed. These symptoms suggest the presence of charcoal disease caused by *Biscogniauxia mediterranea*, an opportunistic fungal infection commonly associated with weakened or stressed trees.

Figure22. Trunk degradation in Cork Oak (*Quercus suber*) observed in Hafir forest

Furthermore, Aleppo pine (*Pinus halepensis*) shows signs of decline, with needles appearing abnormally twisted, reduced in size, and often deformed. Many needles are underdeveloped or atrophied compared to healthy, flexible needles, which typically grow in pairs, are light green in color, and measure between 6 and 13 cm in length. These changes indicate underlying stress or disease (Fig. 23).

Figure23. Symptoms observed on Aleppo pine

2. Importance of phytopathogenic fungi in the study site

Macroscopic and microscopic analyses allowed us to identify Ten fungal species belonging to various genera (Tab.7).

Table 7. Taxonomy of the isolated species

Division	Order	Genus / Species
Ascomycota	Botryosphaeriales	*Dothiorella iberica*
	Pleosporales	*Alternaria alternata*
	Eurotiales	*Penicillium sp.*
	Pleosporales	*Alternaria solani*
	Eurotiales	*Aspergillus sp.*
	Eurotiales	*Aspergillus niger*
	Botryosphaeriales	*Lasiodiplodia exigua*
	Botryosphaeriales	*Diplodiasp.*
	Botryosphaeriales	*Diplodia sapinea*
Mucoromycota	Mucorales	*Rhizopus stolonifer*

After mycological analysis of the 30 samples under various growth conditions, a significant fungal diversity was revealed. Ten fungal species, distributed across several genera including *Alternaria*, *Aspergillus*, *Penicillium*, *Dothiorella*, *Lasiodiplodia*, *Diplodia*, and *Rhizopus*, were isolated during the process.

Macroscopic identificationbased on both surface and reverse colony appearance on culture medium combined with microscopic examination, confirmed the presence of the following species:

- *Alternaria solani*
- *Alternaria alternata*
- *Aspergillus niger*
- *Aspergillus sp.*
- *Penicillium sp.*
- *Dothiorella iberica*
- *Lasiodiplodia exigua*
- *Diplodia sapinea*
- *Diplodia sp.*
- *Rhizopus stolonifer*

Some additional isolates remained unidentified due to their sterility or the absence of reproductive structures.

These results highlight the considerable diversity of fungal species associated with the sampled trees and underscore their potential ecological significance (Fig. 24) (Annex 1).

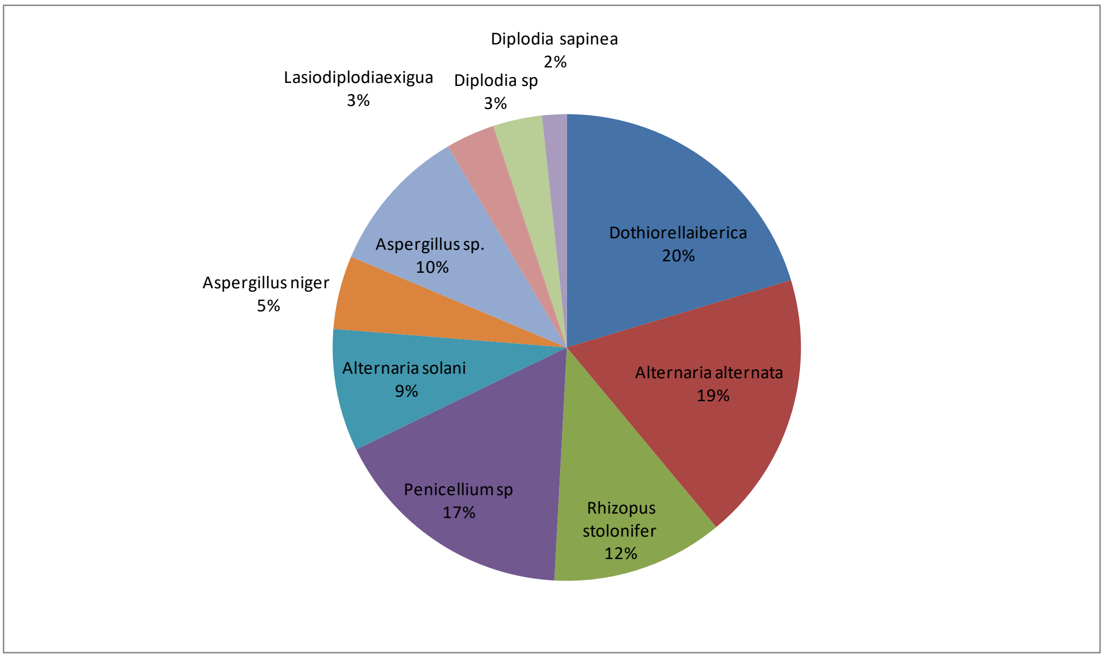

Figure 24. Percentage of species isolated from symptomatic trees.

The graph shows that *Dothiorella iberica* is the most frequently isolated fungus from the sampled trees, with a relative abundance of 20%. This species has been identified in Mediterranean ecosystems and is known to be associated with wood cankers and dieback in oaks and other hardwoods. According to Pérez-Sierra et al. (2018), *D. iberica* has been reported in Mediterranean environments and is linked to decline symptoms and wood necrosis, particularly in oak species. Its prominent presence on Quercus ilex suggests it may contribute significantly to the observed oak decline in the Hafir Forest.

Alternaria alternata and *Alternaria solani* were also frequently isolated, with respective frequencies of 20% and 9%. These cosmopolitan species are commonly found on senescent or stressed plant tissues. A. alternata, in particular, is known as a secondary pathogen that exploits weakened hosts (Maharach *et al.,* 2014), suggesting a role in exacerbating stress-induced decline.

Rhizopus stolonifer was present in 12% of the samples. Although typically saprophytic, it can act as an opportunistic pathogen on damaged or decaying tissues. Similarly, Penicillium species, which occurred in 17% of the samples, are primarily decomposers but may interact with other pathogens or influence host responses.

Two species of Aspergillus were also detected: *Aspergillus niger* (5%) and *Aspergillus sp.* (10%). These fungi are generally saprophytic and thrive in decaying organic matter. However, their consistent presence on declining *Quercus ilex* suggests a possible opportunistic behavior under host stress conditions.

Less frequent but notable isolates include *Lasiodiplodia exigua* and *Diplodia sp.,* each representing 3% of the total isolates. Belonging to the Botryosphaeriaceae family, these fungi are known to cause cankers, shoot blight, and dieback in woody plants, particularly under drought stress or in degraded environments (Slippers & Wingfield, 2007).

Finally, *Diplodia sapinea* was the least frequently detected species, with only 2% occurrence. This fungus is a well-known pathogen of pines, particularly *Pinus halepensis* (Aleppo pine).

The fungal species are distributed among the four host tree species according to their host specificity. Some fungi appear to be generalists, occurring on multiple hosts, while others show a preference or exclusivity for a particular tree species (Fig. 25)

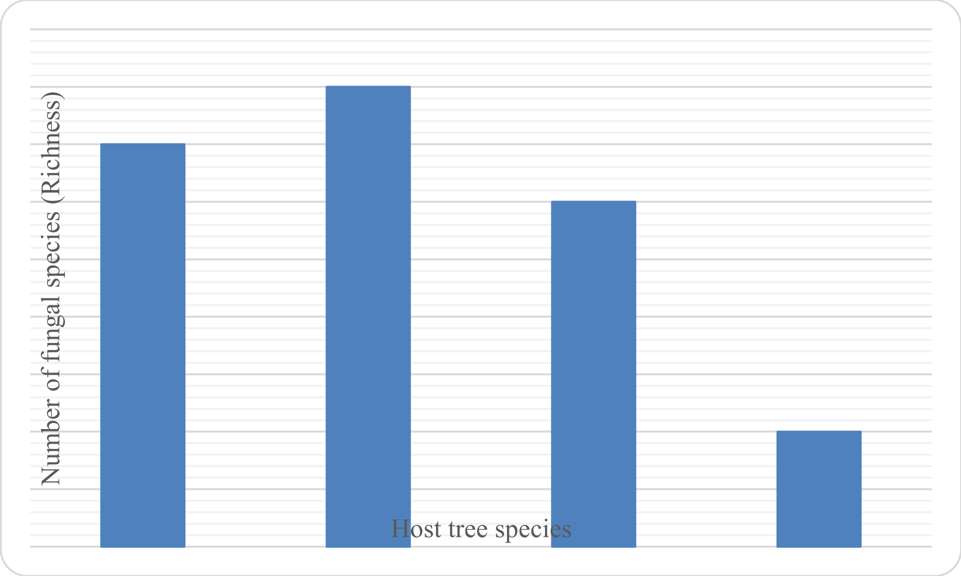

Figure 25. Fungal species richness (number of fungal species) per host tree.

According to this figure showing the species richness (number of different fungal species) for each host tree species, *Quercus suber* exhibits the highest richness (8 species), followed by *Quercus ilex* and *Juniperus oxycedrus*, which show comparable levels (7 and 6 species, respectively). Finally, *Pinus halepensis*, represented by a single sample, shows a lower richness (2 species).

A Shannon index H' = 2.11 indicates a moderate to high diversity: several species are present, and their distribution is relatively balanced (none is completely dominant).

The higher the index, the greater the diversity (richness + evenness).

The results of Fisher's exact test applied to the different fungal species are presented in Figure 26. This analysis was conducted to evaluate the significance of associations between each fungal species and the host tree species (Fig. 26).

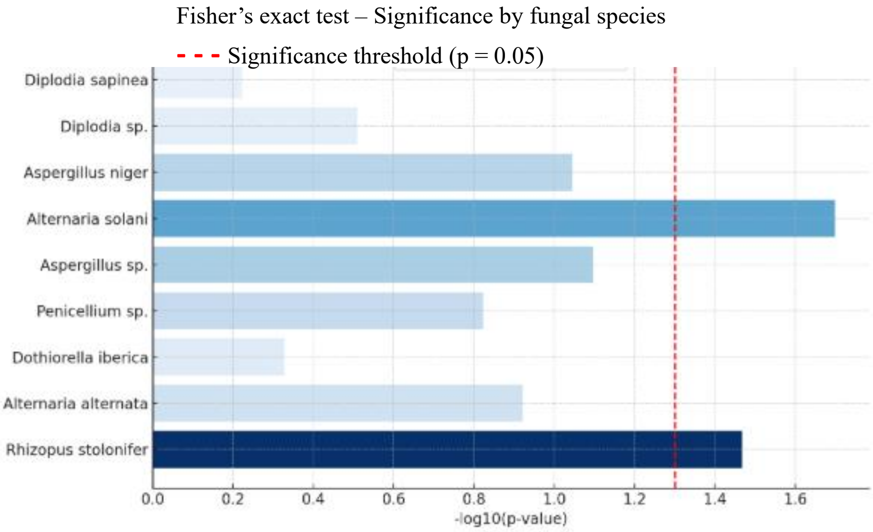

Figure 26. Fisher's exact test – Significance by fungal species

The horizontal axis shows the statistical significance of the test for each fungal species (the longer the bar, the more significant the result).

The red line indicates the significance threshold at $p = 0.05$ (i.e., $-\log_{10}(0.05) \approx 1.3$).

The color intensity of the bars reflects the odds ratio (i.e., the strength of association with the host tree species).

Morphological characteristics of the isolated fungal species

IV.3.3.1. Botryosphaeriales

Botryosphaeria species are cosmopolitan fungi commonly associated with a wide range of woody plants, where they can act as latent pathogens or endophytes (Slippers & Wingfield, 2007). Members of this group are well known for causing cankers, dieback, fruit rot, and leaf spots, especially under stress conditions such as drought, mechanical injury, or poor site quality (Úrbez-Torres, 2011).

1. *Dothiorella iberica*

D. iberica has since been reported on a variety of woody hosts including fruit trees, oaks, and olives (Úrbez-Torres *et al.,* 2004; Phillips *et al.,* 2013). It is considered an opportunistic and endophytic pathogen, frequently associated with shoot dieback, fruit rot, and bark cankers, particularly in hosts under abiotic or biotic stress (Slippers & Wingfield, 2007).

In culture, *D. iberica* colonies grow moderately fast on Potato Dextrose Agar (PDA), initially appearing white to grayish and darkening with age. The fungus forms black, pycnidial conidiomata, often partially immersed in the substrate. Asexual spores (conidia) of Dothiorella species are initially hyaline and aseptate but become brown, thick-walled, and one-septate at maturity. The conidia are typically ellipsoid to ovoid, often with one side slightly flattened. Unlike some other members of the Botryosphaeriaceae, Dothiorella species rarely produce a known sexual morph in culture (Fig. 27).

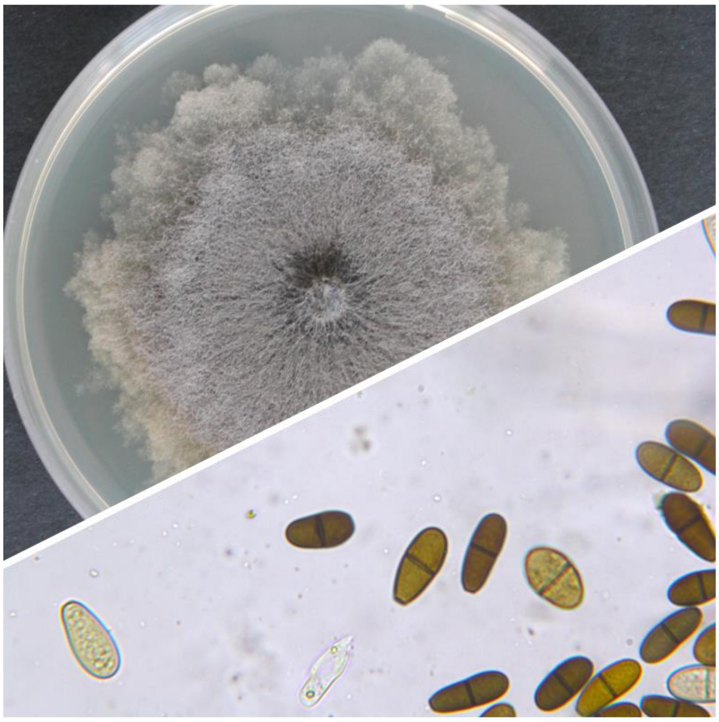

Figure 27. Macroscopic and microscopic observations of *Dothiorella iberica* (Obj. x40)

2. Lasiodiplodia exigua

A diverse range of diseasesincluding cankers, dieback, fruit or root rot, branch blight, stem-end rot, and gummosisare caused by the heterogeneous genus Lasiodiplodia, which is primarily found in tropical and subtropical regions.

Members of the genus Lasiodiplodia exhibit diverse lifestyles on a wide range of host plants, ranging from endophytes, which cause asymptomatic infections in various plant tissues, to pathogens that cause disease, and saprophytes.

Colonies grown on Potato Dextrose Agar (PDA) form moderately dense mycelial mats that are initially white to smoke gray but become greenish gray on both the front and reverse sides. With age, the colony color darkens to a dark slate blue. Pycnidia are produced on PDA after 7 days under these conditions. Colonies reach the edge of a 90 mm Petri dish (85 mm growth) within 3 days in the dark at 30 °C. The cardinal temperature requirements for growth are: minimum 15 °C, optimum 30 °C, and maximum 35 °C, with no growth observed at 10 °C. Isolates produce a pink pigment on PDA at 35 °C (Smahi,2019). Its ability to thrive in warm, dry climates makes it particularly relevant in Mediterranean and semi-arid regions (Pavlic *et al.*, 2004).

3. Diplodia sp.

Species of Diplodia, like other members of the family Botryosphaeriaceae, are known to be pathogens, endophytes, and saprophytes on a wide range of mainly woody hosts (Crous et al., 2006; Slippers & Wingfield, 2007).

Colonies typically appear dark gray to black on culture media such as Potato Dextrose Agar (PDA). They form pycnidia, which are dark, globose asexual fruiting bodies that release conidia. These conidia are initially hyaline and aseptate but become brown and septate with age, often appearing ovoid to ellipsoidal in shape.

Most Diplodia species grow optimally between 25 °C and 30 °C, although some tolerate temperatures up to 35 °C. This thermal flexibility contributes to their widespread occurrence, especially in warm temperate and Mediterranean climates (Slippers & Wingfield, 2007; Smahi, 2019) (Fig. 28).

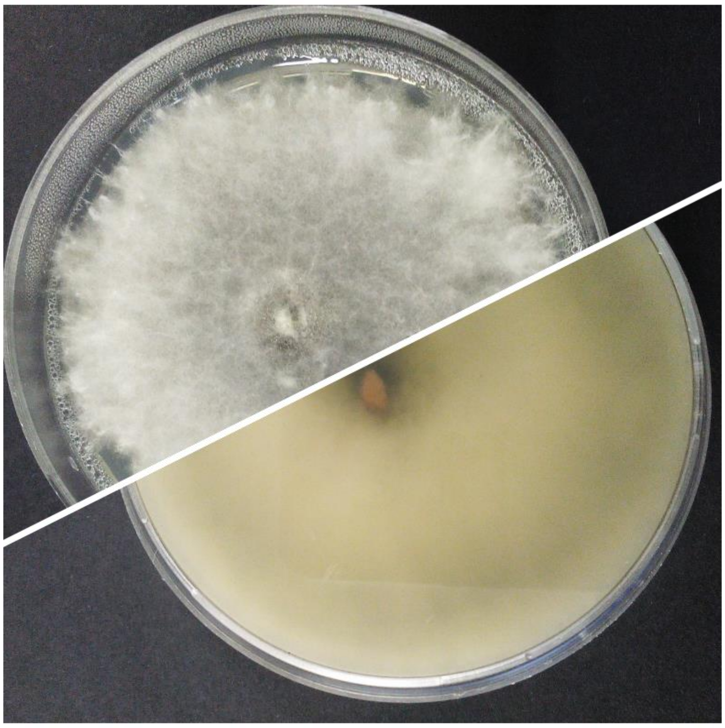

Figure 28. Macroscopic observations of *Diplodia corticola* (Obj. x40)

4. Diplodia sapinea

Diplodia sapinea is a globally distributed fungal species and a well-known endophyte, particularly associated with various tissues of pine trees. While often asymptomatic, this fungus is opportunistic and can switch to a pathogenic phase under environmental stress, leading to disease development when host defenses are compromised. The species was previously known as Diplodia pinea, a name derived from its initial identification on Pinus sylvestris in France.

According to Smahi (2019), *D. sapinea* produces some of the longest conidiomata among members of the Botryosphaeriaceae family. On Potato Dextrose Agar (PDA), isolates exhibit relatively rapid growth. Colonies appear aerial, fluffy, and range from white to grey toward the margin. A faint violet pigmentation is often observed at the colony edge, particularly under low temperature conditions. The reverse side of the colony tends to be dark grey.

Microscopically, the conidia produced within the conidiomata are typically oblong, straight to slightly curved, with an obtuse apex and a truncate base. They are mostly aseptate, though occasionally one to two septa, and rarely three, can be observed. The conidia are dark brown with thick cell walls.

In forest environments, the fungus can spread rapidly, causing shoot blight even in otherwise healthy hosts. Pycnidia (fruiting bodies) may develop quickly on dead shoots and needles. Young, fertilized pine plantations are reported to be particularly susceptible (DSF, 2017).

IV.3.3.2. Pleosporales

1. *Alternaria alternata*

Alternaria alternata is a cosmopolitan, saprophytic, and opportunistic fungal pathogen belonging to the phylum Ascomycota (Woudenberg *et al.,* 2015). It is frequently isolated from diverse environments, including soil, air, decaying plant material, food products, and indoor settings. This species thrives in warm and humid conditions, with cardinal temperatures ranging from 2 °C to 32 °C, and an optimum between 25 °C and 29 °C (Domsch *et al.,* 2007).

On Potato Dextrose Agar (PDA)**,**A. alternata produces dark green, velvety colonies with a distinctly furrowed surface. The reverse side of the colony typically appears dark green to nearly black. It exhibits rapid growth, often covering the entire Petri dish within seven days at 25 °C. The colony is flat, with a texture that ranges from fluffy to woolly. Aerial hyphae are initially hyaline, later turning olive-brown or dark green. The dark pigmentation, often linked to melanin-like compounds, accumulates over time, especially on the colony’s underside.

Under light microscopy, the fungus reveals long, septate mycelial filaments (hyphae) interspersed with asexual reproductive structures (conidia). These conidia are typically ovoid to ellipsoid, muriform, and possess a short, conical to cylindrical beak at one end (Dutron, 2012) (Fig. 29).

2. *Alternaria solani*

Alternaria solani is a pathogenic fungus primarily associated with fruit-bearing plants, within agricultural ecosystems and in natural forest environments**.**

The conidia of A. solani are notably large, typically measuring between 150 and 200 µm in length, from the base to the apex of the beak. The species was first described as the causal agent of early blight in potato (Solanum tuberosum) (Simmons, 2007), a disease of considerable economic importance.

In culture, isolates of A. solani produce low-growing, greenish colonies with a black reverse**,** a pigmentation attributed to the deposition of melanin in the cell walls. The conidiophores are dark brown to black, exhibit sympodial growth, and may be either simple or branched, giving rise to terminal conidial chains (Fig. 29).

Young conidia are initially ovoid, with a broad basal portion and a tapering apex. As they mature, the conidia develop a distinctive elongated beak, resulting in a club-shaped or racket-like morphology. They are multicellular, with both transverse and longitudinal (or oblique) septa, which are considered diagnostic characteristics of the species (Botton *et al.,* 1990).

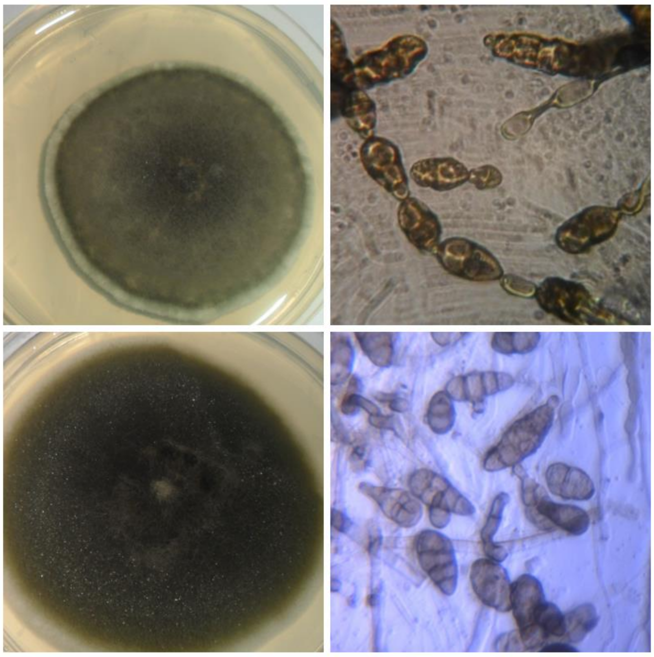

Figure 29. Macroscopic and microscopic observations of *Alternaria alternata* (At the top) and *Alternaria solani* (At the bottom) (Obj. x40)

IV.3.3.3. Eurotiales

1. ***Penicellium sp.***

The genus Penicillium (Order: Eurotiales; Family: Trichocomaceae) is among the most widespread fungal genera in nature. Many species are ubiquitous and capable of colonizing a wide range of habitats. In general, Penicillium species are saprotrophic fungi, commonly found on plant debris, in soil, on decaying organic matter, and on a variety of plant residues (Pitt, 2014).

On solid media such as PDA or MEA, Penicillium colonies are fast-growing, often exhibiting a velvety or powdery texture. Colony color varies depending on the species, ranging from green and blue to yellow or even white. The aerial mycelium gives rise to conidiophores, which bear the diagnostic reproductive structures. Microscopic examination typically reveals spherical to ovoid conidia arranged in characteristic brush-like or columnar formations, contributing to the genus's easily recognizable morphology (Fig. 30).

2. ***Aspergillus sp.***

Aspergillus is a large and taxonomically complex genus, currently comprising approximately 250 species, classified into seven subgenera, which are further subdivided into numerous sections grouping phylogenetically related taxa (Raper & Fennell, 1965; Gams *et al.,* 1985; Geiser *et al.,* 2007). On culture media, Aspergillus colonies are typically fast-growing, displaying a broad spectrum of colors, ranging from white, yellow, yellow-brown, to brown, black, or various shades of green, depending on the species. The conidia are typically unicellular, smooth- or rough-walled, hyaline or pigmented, and are produced in long, dry chains. These chains may appear radiate (divergent) or columnar (compact).

In forest ecosystems, Aspergillus species primarily function as saprotrophs, contributing to the degradation of dead organic matter. However, under stressful conditions such as drought, mechanical injury, or plant senescence, certain species may act as secondary colonizers of weakened tissues (Samson *et al.,* 2014). Their presence in declining trees may indicate opportunistic behavior and potential interactions with other pathogenic fungi (Fig. 30).

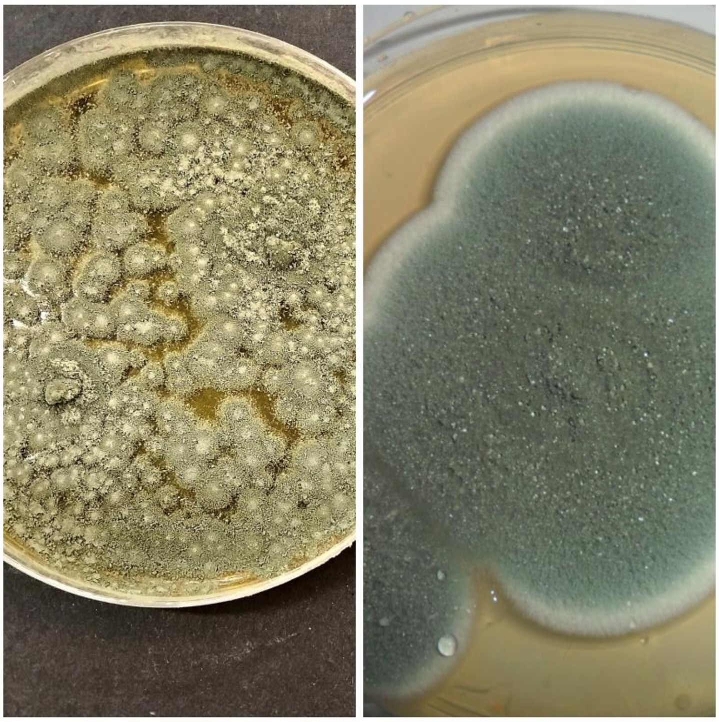

Figure 30. Macroscopic observations of *Penicellium sp.* (On the left) and *Aspergillus* (On the right) (Obj. x40)

3. *Aspergillus niger*

Aspergillus niger is a ubiquitous filamentous fungus with a cosmopolitan distribution. It is known for its remarkable ecological plasticity, being isolated from diverse environments such as agricultural soils, forest ecosystems, decaying vegetation, marine environments. From a morphological standpoint, A. niger forms colonies on PDA that are black to dark brown with dense conidial heads and a rapid growth rate. The conidiophores are long, smooth-walled, terminating in globose vesicles covered with biseriate chains of black, rough-walled conidia.

IV.3.3.4. Mucorales

1. *Rhizopus stolonifer*

Rhizopus species act as strict wound parasites, meaning they can only infect host tissues through wounds, abrasions, or bruises created during harvesting, handling, or by insect activity. This infection strategy is likely due to their inability to produce certain extracellular enzymes, such as cutinases or esterases, which are typically required for direct penetration of intact plant surfaces (Nguyen-The & Chamel, 1991).

Rhizopus stolonifer grows rapidly on standard culture media such as Potato Dextrose Agar (PDA), forming fluffy, cotton-like colonies. Initially white, the mycelium gradually turns gray to black as abundant sporangia develop on the surface (Alexopoulos *et al.,* 1996). The vegetative mycelium is coenocytic (non-septate) and consists of three distinct types of hyphae: Stolons, which grow horizontally along the substrate surface; Rhizoids, which extend downward to anchor the colony into the medium; Sporangiophores, which arise directly opposite the rhizoids and terminate in spherical black sporangia, responsible for asexual reproduction (Fig. 30).

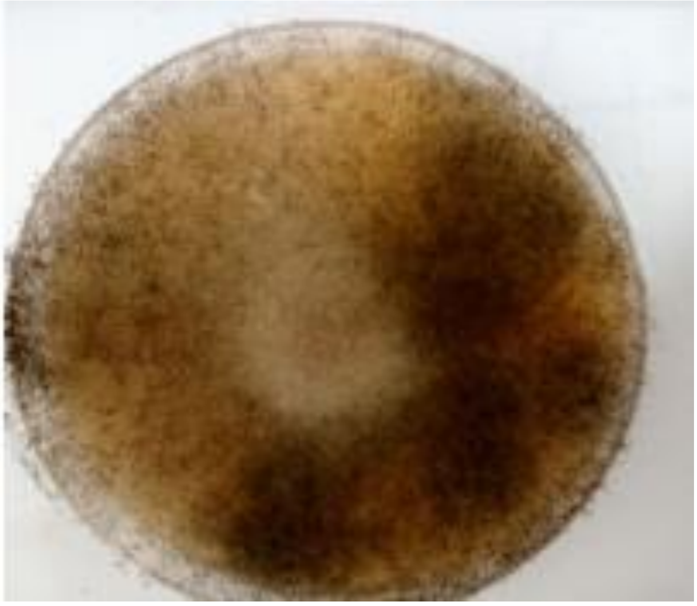

Figure 31. Macroscopic observations of *Rhizopus stolonifer* (Obj. x40)

1. Analyzing Antagonism: Test Results

The antagonism test was conducted in vitro using the dual culture method on Potato Dextrose Agar (PDA) medium, incubated at 25 °C. Two fungal strains, *Trichoderma sp.* and *Diplodia sp.*, were inoculated opposite each other on a single Petri dish to observe their interactive dynamics over a defined period.

On the first day of the experiment, both fungal species exhibited comparable initial growth, reaching approximately 0.5 cm in both vertical and horizontal dimensions. By the second day, differences in growth rates became apparent: Trichoderma sp. expanded to 0.4 cm in height and 0.2 cm in width, while Diplodia sp. showed comparatively limited growth, measuring 0.2 cm in height and 0.1 cm in width.

By day three, visual observations revealed that *Trichoderma sp.* exhibited signs of antagonistic activity, including directional growth toward the pathogen and the formation of potential inhibition zones.

At the end of the incubation period, Trichoderma sp. had clearly dominated the Petri dish, occupying the majority of the substrate, whereas the growth of *Diplodia sp.* was restricted to a minimal area. This suggests a strong inhibitory effect, likely resulting from the secretion of antifungal metabolites disrupting the pathogen’s development. The interaction led to more than 50% inhibition of the pathogen’s mycelial growth, confirming the effective antagonistic potential of *Trichoderma sp.* (Fig. 32).

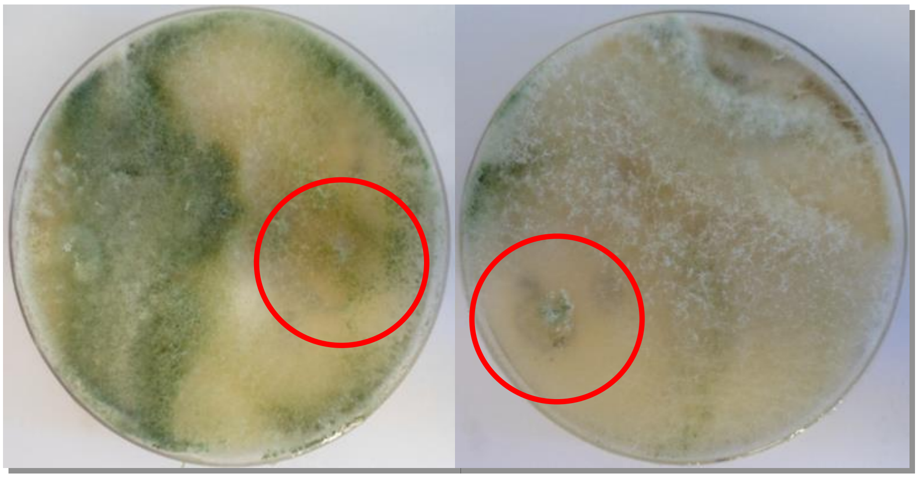

Figure 32. Evaluation of antagonistic activity between *Trichoderma sp.* and *Diplodia sp.*

Conclusion

Cryptogamic diseases represent an increasing threat to forest ecosystems, particularly in the Mediterranean region, where environmental stressors such as drought, elevated temperatures, and anthropogenic disturbances create favorable conditions for pathogenic outbreaks. This study provided valuable insights into the declining health of Hafir Forest (Tlemcen, Algeria), emphasizing the significant role of phytopathogenic fungi in the deterioration of several native tree species, especially holm oak (*Quercus ilex*) and cork oak (*Quercus suber*).

Through an integrated approach combining field surveys, symptom analysis, and mycological identification, ten fungal species were isolated, many of which belong to genera known for their pathogenic potential, including *Dothiorella*, *Diplodia*, *Lasiodiplodia*, *Alternaria*, and *Aspergillus*. The results revealed a moderate to high fungal diversity (Shannon index $H' = 2.11$) and underscored significant associations between certain fungal species and their host trees. *Dothiorella iberica*, the most frequently isolated species, appears to play a central role in oak decline, particularly in combination with environmental stressors such as drought and heat.

Several of the isolated species are associated with severe forest pathologies. Members of the Botryosphaeriaceae family such as *Dothiorella iberica*, *Lasiodiplodia exigua*, and *Diplodia sapinea* are known to cause cankers, shoot blight, and dieback in a wide range of tree species, especially under drought stress. Although often considered opportunistic, species of *Alternaria* and *Aspergillus* may contribute to tissue degradation and act as secondary pathogens under favorable conditions. Likewise, *Rhizopus stolonifer*, typically a saprophyte, may colonize weakened tissues and exacerbate disease symptoms.

This study also opens important perspectives for future research, notably through the use of molecular tools to improve the accuracy of pathogen identification and the expansion of surveys to other forest ecosystems in northwestern Algeria. Enhancing disease monitoring and implementing preventive strategies will be essential to safeguarding the resilience and sustainability of Mediterranean forest ecosystems in the face of climate change and the emergence of new pathogenic threats.

References

A

Acácio, V. (2009). *The dynamics of cork oak systems in Portugal: The role of ecological and land use factors* (Doctoral dissertation, Wageningen University).

Ainsworth, G. C. (1976). *Introduction to the History of Mycology*. Cambridge University Press.

Agrios, G. N. (2005). *Plant pathology* (5th ed.). Elsevier Academic Press.Alexopoulos, C. J., Mims, C. W., & Blackwell, M. (1996). *Introductory mycology* (4th ed.). John Wiley & Sons.

Azerizer, A., &Mansseri, A. (2003). *Usinabilité et qualification des états de surface du chêne zeen algérien (Quercus canariensisWilld.): Cas de la station de Beni Ghobri,*

B

Bart, T. P., Boekhout, T., Crous, P. W., Robert, V., &Verkley, G. J. M. (2016).*Fungal Biodiversity*. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Ballesteros, M., Peguero-Pina, J. J., Galmés, J., &Flexas, J. (2023). *Physiological and anatomical strategies underpinning the drought tolerance of holm oak (Quercus ilex L.) across its distribution range*. *TreePhysiology*, 43(3), 384–398.

Belhoucine, K. (2008). Contribution à l'étude des champignons entomopathogènes des sols de la région de Tlemcen. *Mémoire de Magister*, Université Abou Bekr Belkaid Tlemcen, Algérie.

Belkhir, M., Gharbi, M., &Derridj, A. (2011). *Approche méthodologique de l'évaluation de l'état de dépérissement du chêne-liège en Algérie*. Revue des Régions Arides, 27(2), 116–122.

Benabadji, N., Letreuch-Belarouci, N., & Bouazza, M. (2007). Biodiversity and ecology of *Quercus canariensis* forests in northwestern Algeria. *Acta BotanicaMalacitana*, 32, 145–158.

Benabdallah, S., & Amrani, M. (2014). *Contribution à l'étude du climat et des ressources en eau dans la région de Tlemcen (Algérie)*. Revue des Sciences et de la Technologie, 26, 70–77.

Bendjebbar, K., Belhoucine-Guezouli, L., Bouandas, A., Boughalem, M., Bonifácio, L., & Henriques, J. (2023). Characterization of cork oak infection by *Biscogniauxia mediterranea* in two cork oak forests of Occidental Algeria: Djebel Saadia (W. Relizane) and Hafir (W. Tlemcen). *Revue Agrobiologia*, 13(1), 3348–3355.

Benhachem, A., &Harrache, L. (2020). *Évaluation de la qualité physico-chimique et bactériologique des eaux souterraines utilisées comme eau de boisson dans la région de Tlemcen (Algérie)*. Larhyss Journal, 41, 179–194.

Benny, G. L., O'Donnell, K., &Huhndorf, S. M. (2001). Phylogenetic and taxonomic overview of the Zygomycota.

Besnard, G., &Berville, A. (2000). Multiple origins for Mediterranean olive (*Olea europaea* L. subsp. *europaea*) based upon mitochondrial DNA polymorphisms. *Comptes Rendus de l'Académie des Sciences - Series III - Life Sciences*, 323(2), 173–181.

Bhunjun, C. S., et al. (2023). Challenges and update on fungal endophytes: Classification, definition, diversity, ecology, evolution and functions. *Fungal Diversity*. https://doi.org/10.1007/s13225-023-00533-6

Blancard, D. (2015). INRA. Diagno-Lég Conidies. *EPHYTIA*.

Bouhraoua, R. T. (2003). *Situation sanitaire de quelques forêts de chêne-liège de l'Ouest algérien : Étude particulière des problèmes posés par les insectes* (Thèse d'état). Département de foresterie, Faculté des sciences, Université de Tlemcen.

Bouhraoua, R., Smahi, R., &bendjabbar, H. (2015). Diversité fongique dans les forêts de cheneliege. Algérie Forestière, 5(2), 33-47.

Breton, C. M., Villemur, P., & Berville, A. J. (2017). The sporophytic self-incompatibility mating system is conserved in *Olea europaea* subsp. *cuspidata* and *O. e. europaea*. *Euphytica*, 213(1), 22.

Brockerhoff, E. G., et al. (2013). *Diplodia sapinea* and pine health: Global implications.*Forest Ecology and Management*, 289, 1–5.

Bowman, S. M., & Free, S. J. (2006). The structure and synthesis of the fungal cell wall. *BioEssays*, 28(8), 799–808.

C

Carris, L. M., Little, C. R., & Stiles, C. M. (2012). Introduction to fungi. *Plant Health Instructor*, 1–25. https://doi.org/10.1094/PHI-I-2012-0426-01

Crisosto, C., Ferguson, L., & Nanos, G. (2011). Olive (*Olea europaea* L.). In Y. Yahia (Ed.), *Postharvest biology and technology of tropical and subtropical fruits* (Vol. 2, pp. 63–87e). Elsevier.

Crous, P. W., Slippers, B., Wingfield, M. J., Rheeder, J., Marasas, W. F. O., et al. (2006). Phylogenetic lineages in the Botryosphaeriaceae. *Studies in Mycology*, 55, 235–253. https://doi.org/10.3114/sim.55.1.235

D

Dahmani, M. (1997). *Le chêne vert en Algérie: Syntaxonomie, phyto-écologie et dynamique des peuplements* (Doctoral dissertation). Université des Sciences et de la Technologie Houari Boumediene (USTHB), Alger, 330 p.

Desprez-Loustau, M. L., Robin, C., Buée, M., Courtecuisse, R., Garbaye, J., Suffert, F., Sache, I., & Rizzo, D. M. (2007). The fungal dimension of biological invasions. *Trends in Ecology & Evolution*, 22(9), 472–480.

Dijksterhuis, J. (2013). Fungal spores: Highly variable and stress-resistant vehicles for distribution and spoilage. *Food Microbiology*, 38, 2–11.

Domsch, K. H., Gams, W., & Anderson, T.-H. (2007). *Compendium of soil fungi* (2nd ed.). IHW Verlag.

D S. F. (2017). *Le Sphaeropsis des pins*. Département de la Santé des Forêts.

Dubey, S. C., & Pandey, K. K. (2017). Fungal diseases of forest trees: current status and future management. In: *Recent Advances in Forest Microbiology* (pp. 175–202). Springer.

Dutron, J. (2012). *Fiches mycologiques de terrain*. Éditions Persée.

E

Elmi, S. (1970). Rôles des accidents décrochants de direction SSW–NNE dans la structure des monts de Tlemcen (Ouest Algérie). *Bulletin de la Société d'Histoire Naturelle de l'Afrique du Nord*, 61, 3–8.

G

Gest, H. (2004). The discovery of microorganisms by Robert Hooke and Antoni van Leeuwenhoek, Fellowsofthe RoyalSociety. *Notes and Records of theRoyal Society of London*, 58(2), 187–201. https://doi.org/10.1098/rsnr.2004.0055

Giachero, M. L., Declerck, S., & Marquez, N. (2022). *Phytophthora* root rot: Importance of the disease, current and novel methods of control. *Agronomy*, 12(3), 610.

Goheen, E. M., &Otrosina, W. J. (1998). Characteristics and consequences of root diseases in western coniferous forests. In: *Frankel, S. J., Tech. Coord. Proceedings of the Symposium on the Sustainability of Forest Ecosystems*, Gen. Tech. Rep. PSW-GTR-168. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. pp. 76–81

Gomdola, D. (2022). *Fungal interactions and their ecological significance*. Fungal Diversity, 112, 125–139.

Gonthier, P., &Garbelotto, M. (2013).*The importance of hybridization in the evolution of forest pathogens and pests*. Biological Reviews, 88(3), 939–955.

Graves, R.(1955).*TheGreekmyths*.PenguinBooks.

Green, P. S. (2002). A revision of *Olea* L. (*Oleaceae*). *Kew Bulletin*, 54(1), 91–140.

H

Haffaf S. 2011. Contribution to the study of cork oak entomofaune in the forest of Zerifet.

Hansen, E. M., & Goheen, E. M. (2000). *Phellinus weirii* and other native root pathogens as determinants of forest structure and process in Western North America. *Annual Review of Phytopathology, 38*, 515–539.

Hardham, A. R. (2005).*Phytophthora cinnamomi*. Molecular Plant Pathology, 6(6), 589–604.

Hibbett, D., Abarenkov, K., Kõljalg, U., Öpik, M., Chai, B., Cole, J., Wang, Q., Crous, P., Robert, V., Helgason, T., Herr, J. R., Kirk, P., Lueschow, S., O'Donnell, K., Nilsson, R. H., Oono, R., Schoch, C., Smyth, C., Walker, D. M., Porras-Alfaro, A., Taylor, J. W., & Geiser, D. M. (2016). Sequence-based classification and identification of fungi. *Mycologia*, 108(6),

I

IPCC. (2021). *Climate change 2021: Impacts, adaptation and vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.*Cambridge UniversityPress.

Ikermoud, N. (2000). *La forêt algérienne: Contraintes et perspectives.* Ministère de l'Agriculture, Algérie.

J

Jampilek, J. (2022). *Fungi: Diversity, interactions with plants and humans, and potential applications*. Microorganisms, 10(2), 313.

Jules, E. S., Kauffman, M. J., Ritts, W. D., & Carroll, A. L. (2014).Spread of an invasive pathogen over a variable landscape: A nonnative root rot on Port Orford cedar. *Ecological Applications, 12*(2), 541–556.

K

Kouider, M., Bouteldja, S., &Djellouli, Y. (2019). *Contribution à l'étude de la flore et de la diversité des écosystèmes forestiers de la région de Tlemcen (Nord-Ouest Algérien)*. Revue des BioRessources, 9(1), 87–98.

Kaid Slimane, A.(1999). Etude stratigraphique des monts de Tlemcen. Annales de Géologie Algérienne, 11(2), 55-71.

L

Langwig, K. E., White, J. P., Parise, K. L., Kaarakka, H. M., Redell, J. A., DePue, J. E., Scullon, W. H., Foster, J. T., Kilpatrick, A. M., & Hoyt, J. R. (2021). Mobility and infectiousness in the spatial spread of an emerging fungal pathogen. *Journal of Animal Ecology*, 90(5), 1134–1141.

Lee, S. C., Ni, M., Li, W., Shertz, C., & Heitman, J. (2010). The evolution of sex: a perspective from the fungal kingdom. *Microbiology and MolecularBiologyReviews*, 74(2), 298–340.

Letreuch-Belarouci, A. (2010). *Caractérisation structurale des subéraies du Parc National de Tlemcen, régénération naturelle et gestion durable* [Doctoral dissertation, Université de Tlemcen, Département des Sciences Forestières]. 211 p.

Li, B., Mengiste, T., &Thomma, B. P. H. J. (2022).Infection strategies of fungal plant pathogens: From basic biology to molecular mechanisms. *Annual Review of Phytopathology, 60*, 23–45.

Linnaeus,C.(1753).*Speciesplantarum*.Laurentii Salvii.

López-Ballesteros, A., Rodríguez-Caballero, E., Moreno, G., Escribano, P., Hereș, A. M., & Curiel-Yuste, J. (2023). Topography modulates climate sensitivity of multidecadal trends of holm oak decline. *Science of the Total Environment*, 893, Article 164720.

Louise, F., Müller, M. M., Hantula, J., &Kaitera, J. (2010).*Asexual spore production and dispersal of fungal forest pathogens: Ecological and epidemiological implications*. Forest Pathology, 40(2), 93–99.

M

Maazaouz, R. (2022). *Contribution à l'étude des écosystèmes forestiers du massif de Hafir (Tlemcen): Diagnostic, fonctionnement et perspectives de gestion durable* [Mémoire de Magister, Université Abou Bekr Belkaid Tlemcen, Algérie].

Maharachchikumbura, S. S. N., Hyde, K. D., Groenewald, J. Z., Xu, J., Crous, P. W., & others. (2014). Alternaria species: Biology, ecology and pathology. *Fungal Diversity, 68*(1), 149–162.

Manion, P. D. (1991). *Tree disease concepts* (2nd ed.). Prentice-Hall.

Manion, P. D., & Lachance, D. (Eds.). (1992). *Forest decline concepts*. APS Press.

Magyar, D., Tóth, Á., &Bidló, A. (2022). *Forest decline and fungal pathogens: Current understanding and future perspectives*. Forests, 13(5), 689.

McAree, J. C. (1975). *Silvicultural methods for the control of cryptogamic diseases*. European Journal of Forest Pathology, 5(1), 1–12.

Mechergui, K., Altamimi, A. S., Jaouadi, W., Naghmouchi, S., &Ammari, Y. (2021). Effect of climate change on the spatial distribution and cork production of *Quercus suber* L., the risk of exclusion by the Aleppo pine expansion, and management practices to protect *Q. suber* habitat: A review. *Ecological Questions, 49*(1), 2–10.

Mekonene, A., & Kebede, T. (2024). *Recent advances in the use of biocontrol agents against plant pathogens*. Journal of Agricultural Science and Innovations, 12(1), 45–60.

Micheli,A.(1729).*Novaplantarumgenera*.Florence:BernardiPaperini

Möhlmann, L. (2011).*The costs and benefits of sexual versus asexual reproduction in fungi*. Master's thesis, Utrecht University, The Netherlands.

Mostefaï, N. (2011). Hafir (Algeria). *Alauda, 79*(3).

N

Nguyen-The, C., & Chamel, A. (1991). Enzymatic degradation of isolated plant cuticles and nectarine fruit epidermis by culture filtrates of phytopathogenic fungi. *Agronomie, 11*, 101–108.

O

Ogunsiji, A. O., & Odusanya, O. O. (2020). *Principles of plant disease management*. In *Introduction to Plant Pathology* (pp. 89–102).

P

Pausas,J.G.,&Millán,M.M.(2019).Greeningandbrowninginaclimatechangehotspot :TheMediterraneanBasin.*Land*,**8**(12),206.https://doi.org/10.3390/land8120206

Pavlic, D., Slippers, B., Coutinho, T. A., Gryzenhout, M., & Wingfield, M. J. (2004). Characteristics and distribution of *Botryosphaeriaceae* fungi associated with dieback of trees in Mediterranean regions. *Forest Pathology, 34*(4), 243–257.

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 their role as biocontrol agents. *Frontiers in Microbiology, 12*, 678356.

Pérez-Sierra, A., León, M., García-Jiménez, J., &Abad-Campos, P. (2018). *Dothiorellaiberica* associated with decline of Mediterranean oaks. *Forest Pathology, 48*(6), e12480.

Phillips, A. J. L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M. J., Groenewald, J. Z., & Crous, P. W. (2013). The *Botryosphaeriaceae*: Genera and species known from culture. *Studies in Mycology, 76*, 51–167.

Popier, A. (1985). *La vie mystérieuse des champignons*. *Publications de la Société Linnéenne de Lyon, 54*(7), 1644

Prospero, S., & Cleary, M. (2017). Effects of host variability on the spread of invasive forest diseases. *Forest Pathology, 47*(5), e12352.

Porta, N., Capretti, P., Thomsen, I. M., Kasanen, R., Hietala, A. M., & Solheim, H. (2008). Forest pathogens with higher damage potential due to climate change in Europe. *Canadian Journal of Plant Pathology*, 30(2), 177–195

Q

Quézel, P. and Medail, F. 2003. Ecologie et biogéographie des forêts du bassin méditerranéen. Lavoisier, Paris, 592 pp

R

Raitelaitytė, K., Rutkauskas, A., Radzijevskaja, J., Žukauskienė, J., Markovskaja, S., &Paulauskas, A. (2016). The fungal pathogens causing diseases in pines. *Biologija, 62*(4), 177–180.

Rego, L., Mota, S., Torres, A., Pinto, C., Cravo, S., Silva, J. R. e., Páscoa, R. N. M. J., Almeida, A., Amaro, F., Pinho, P. G., & Others. (2023). *Quercus suber* bark as a sustainable source of value-added compounds: Experimental studies with cork by-products. *Forests, 14*(3), 543.

Rey, M.-D., Labella-Ortega, M., Guerrero-Sánchez, V. M., Carleial, R., Castillejo, M. Á., Rodríguez-Franco, A., Buggs, R. G., Ruggieri, V., &Jorrín-Novo, J. V. (2022). A first draft genome of holm oak (*Quercus ilex* L.), the most representative species of the Mediterranean forest and the Spanish agrosilvopastoral ecosystem "dehesa." *Frontiers in Forests and Global Change, 5*, 830605.

Rodà, F., Retana, J., Gracia, C. A., & Bellot, J. (1999). *Ecological Bases of Mediterranean Forest Management: Proceedings of the International Conference held in Barcelona, Spain, 20–22 October 1997*. Springer-Verlag.

Riley, R., Salamov, A., Hibbett, D., & Grigoriev, I. (2011). Phylogeny and comparative genome analysis of Basidiomycete fungi. *Mycologia, 103*(2), 333–346.

Riquelme, M. (2018). Tip growth in filamentous fungi: A road trip to the apex. *Annual Review of Microbiology*, 72, 559–577.

S

Sánchez, M. E., Trapero, A., & Pérez-De-Luque, A. (2015). Cryptogamic diseases in Holm oak forests: A critical threat to Mediterranean ecosystems. *Forest Pathology, 45*(5), 345–356.

Sándor, D. (2020). Spore dispersal strategies in fungi: Diversity and ecological roles. *Acta Microbiologica et ImmunologicaHungarica*, 67(3), 165–180.

Senanayake, I. C., Pem, D., Rathnayaka, A. R., Wijesinghe, S. N., Tibpromma, S., Wanasinghe, D. N., Phookamsak, R., Kularathnage, N. D., Gomdola, D., Harishchandra, D., Dissanayake, L. S., Xiang, M., Ekanayaka, A. H., McKenzie, E. H. C., Hyde, K. D., Zhang, H., & Xie, N. (2022). Predicting global numbers of teleomorphic ascomycetes. *Fungal Diversity, 114*, 237–278.

Shubhrata, R. (2005).*Fungi: Morphology, Reproduction and Classification*. New Delhi: Discovery Publishing House.

Simmons, E. G. (2007). *Alternaria: An identification manual*. CBS Fungal Biodiversity Centre.

Smahi, H. (2019). *Étude des maladies cryptogamiques du chêne-liège (Quercus suber L.) dans les forêts de l'ouest algérien: Cas de la forêt de Hafir (W. Tlemcen) et M'sila (W. Oran)* [Master'sthesis, University of Tlemcen].

Slippers, B., & Wingfield, M. J. (2007). Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. *Fungal Biology Reviews, 21*(2–3),

Stajich, J. E., Berbee, M. L., Blackwell, M., Hibbett, D. S., James, T. Y., Spatafora, J. W., & Taylor, J. W. (2009). The fungi. *CurrentBiology, 19*(18), R694–R698.

T

Taibi, A. (2012). *Contribution à une étude descriptive des peuplements à chêne zéen (Quercus fagineaLamk.) dans les forêts de Zariffet et Hafir (Wilaya de Tlemcen)* [Mémoire d'ingéniorat en foresterie, Université de Tlemcen].

Thomma, B. P. H. J. (2003). *Alternaria* spp.: From general saprophyte to specific parasite. *Molecular Plant Pathology, 4*(4), 225–236.

Timon, A. M., Kocsubé, S., Vágvölgyi, C., & Krizsán, K. (2013). Morphological and molecular comparison of sexual and asexual spores of fungi. *Acta Microbiologica et ImmunologicaHungarica*, 60(4), 399–420.

Tjosvold, S. A. (2018).*The Plant Disease Triangle: A Plant Pathologist's Tool for Understanding Plant Diseases and Their Development*. University of California Agriculture and Natural Resources

U

Úrbez-Torres, J. R., Leavitt, G. M., Guerrero, J. C., Guevara, J., &Gubler, W. D. (2007). Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. *Plant Disease, 91*(6), 772. https://doi.org/10.1094/PDIS-91-6-0772

Úrbez-Torres, J. R. (2011). The status of *Botryosphaeriaceae* species infecting grapevines. *PhytopathologiaMediterranea, 50*(Supplement), S5–S45.

V

Vargas, P., Muñoz Garmendia, F., Hess, J., &Kadereit, J. (2000). *Olea europaea* subsp. *guanchica* and subsp. *maroccana* (Oleaceae), two new names for olive tree relatives. *Anales delJardínBotánico de Madrid, 58*(2), 360–361.

Viegas, C., Dias, M., Carolino, E., & Sabino, R. (2021). Culture media and sampling collection method for *Aspergillus* spp. assessment: Tackling the gap between recommendations and the scientific evidence. *Atmosphere, 12*(1), 23.

Y

Liu, Y., & He, F. (2019).*Soil water content mediates the competition between tree seedlings and herbaceous vegetation in a temperate forest understorey*. Forests, *10*(5), 418.

Younsi, S. E., Adjami, Y., Ghanem, R., Bouchaib, B., &Ouakid, M. L. (2021). Impact of different factors degrading cork oak stands in the Mediterranean region: A case study from Algeria. *Journal of Forest Science, 67*(12), 570–581.

W

Wallen, R. M., & Perlin, M. H. (2018). An overview of the function and maintenance of sexual reproduction in dikaryotic fungi. *Frontiers in Microbiology*, 9, 503.

Wang, Y.-W., McKeon, M. C., Elmore, H., Hess, J., Harrow, L., Gonçalves, S. C., Hull, C. M., & Pringle, A. (2023). Invasive Californian death caps develop mushrooms unisexually and bisexually. *Nature Communications*, 14, 6560.

Winder, R. S., & Shamoun, S. F. (2006). Forest pathogens: Friend or foe to biodiversity? *Canadian Journal of Plant Pathology, 28*(S1), S221–S227.

Wösten, H. A. B., &Dijksterhuis, J. (2013). Fungi and their biological interaction with insects. *Fungal Biology Reviews*, 27(3–4), 112–122.

Woudenberg, J. H. C., Groenewald, J. Z., Binder, M., & Crous, P. W. (2015). *Alternaria*redefined. *Studies in Mycology, 82*, 1–165.

Z

Zerizer, A., &Mansseri, A. (2003). *Le Chêne zeen dans les forêts algériennes: Écologie, répartition et perspectives de valorisation*. Revue des Régions Arides, Special Issue (17), 311–315.

Zerrouki, M., Belkhir, M., Boudyach, E. H., & El Bouami, F. (2022). *État sanitaire du chêne-liège (Quercus suber L.) dans la forêt de M'Sila (Tlemcen, Algérie)*. Algerian Journal of AridEnvironment, 12(2), 1–12.

Annex

Samples	Samples	Samples	Samples	Samples	Samples	Samples
S1	10/11/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Dothiorellaiberica ;Alternaria alternata ; Rhizopus stolonifer*
S2	10/11/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Penicellium sp. ; Alternaria solani*
S3	10/11/2024	Hafirforest	Juniperusoxycedrus	Leaf/Branch	+	*Penicelliumsp. ;Alternaria alternata ;Rhizopus stolonifer*
S4	10/11/2024	Hafirforest	Quercus suber	Leaf/Branch	+	*Aspergillus niger ;Dothiorellaiberica ; Alternaria alternata*
S5	10/11/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Dothiorellaiberica ; Aspergillus sp. ;Rhizopus stolonifer*
S6	10/11/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Alternaria alternata ;Penicellium sp.*
S7	10/11/2024	Hafirforest	Quercus suber	Leaf/Branch	+	*Lasiodiplodiaexigua ; Alternaria solani*
S8	10/11/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Rhizopus stolonifer*
S9	10/11/2024	Hafirforest	Quercus suber	Leaf/Branch	-	-
S10	10/11/2024	Hafirforest	Juniperusoxycedrus	Leaf/Branch	+	*Alternaria alternata ; Aspergillus sp.*
S11	10/11/2024	Hafirforest	Quercus ilex	Leaf/Branch	-	-
S12	10/11/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Dothiorellaiberica ;Alternaria solani*
S13	10/11/2024	Hafirforest	Juniperusoxycedrus	Leaf/Branch	-	-
S14	10/11/2024	Hafirforest	Quercus suber	Leaf/Branch	+	*Penicellium sp. ;Dothiorellaiberica*
S15	10/11/2024	Hafirforest	Juniperusoxycedrus	Leaf/Branch	+	*Penicellium sp. ; Aspergillus niger*
S16	10/11/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Penicellium sp. ; Aspergillus sp*
S17	10/11/2024	Hafirforest	Juniperusoxycedrus	Leaf/Branch	+	*Alternaria alternata ;Penicellium sp. ; Aspergillus niger*
S18	01/12/2024	Hafirforest	Quercus suber	Leaf/Branch	+	*Dothiorellaiberica ; Diplodia sp. ; Aspergillus sp.*
S19	01/12/2024	Hafirforest	Quercus suber	Leaf/Branch	+	*Aspergillus niger ;Dothiorellaiberica ;Penicellium sp.*
S20	01/12/2024	Hafirforest	Quercus suber	Leaf/Branch	+	*Dothiorellaiberica ; Alternaria alternata ;Diplodia sp.*
S21	01/12/2024	Hafirforest	Quercus suber	Leaf/Branch	+	*Lasiodiplodiaexigua ; Dothiorellaiberica*
S22	01/12/2024	Hafirforest	Quercus suber	Leaf/Branch	+	*Dothiorellaiberica ; Alternaria alternata*
S23	01/12/2024	Hafirforest	Quercus suber	Leaf/Branch	+	*Dothiorellaiberica ; Diplodia sp.*
S24	01/12/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Alternaria alternata ; Penicellium sp.*
S25	01/12/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Alternaria solani ; Aspergillus sp.*
S26	01/12/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Dothiorellaiberica ; Rhizopus stolonifer*

S27	01/12/2024	Hafirforest	Quercus ilex	Leaf/Branch	−	-
S28	01/12/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Alternaria alternata ; Aspergillus sp. ;Rhizopus stolonifer*
S29	01/12/2024	Hafirforest	Pinus halepensis	Leaf/Branch	+	*Diplodia sapinea ; Alternaria alternata*
S30	01/12/2024	Hafirforest	Quercus ilex	Leaf/Branch	+	*Alternaria solani ;Penicellium sp. ;Rhizopus stolonifer*