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**Department of natural sciences and life**

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Presented by:
**Siham Heddar**

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## Theme

# Antifungal activity of essential oils extracted from the Algerian flora

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**Jury:**

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| Pr. | Khadidja Boukharouba | Pr | University of Biskra | President |
| Dr. | Tarek Benmeddour | MCA | University of Biskra | Supervisor |
| Mme. | Fedjria Yaakoub | MAA | University of Biskra | Examiner |

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**List of contents**

**Acknowledgement**

**Dedication**

**List of contents**

List of tables ..... **I**

List of figures ..... **II**

List of abbreviations ..... **III**

Introduction ..... **1**

**Chapter 01: Fungi**

1.1.Fungi ..... **3**

1.1.1. Definition ..... 3

.1.1.2 Classification ..... 3

1.1.2.1. Chytridiomycetes ..... 3

1.1.2.2. Zygomycetes ..... 3

1.1.2.3. Ascomycetes ..... 4

.1.1.2.4 Basidiomycetes ..... 4

.1.1.2.5 Deuteromycetes ..... 4

.1.1.3 Role of fungi in nature ..... 5

**Chapter 02: Essential oils**

.2.1Essential Oil ..... **7**

.2.1.1 Definition ..... 7

.2.1.2 Location and Place of Synthesis ..... 7

.2.1.3 Physical properties ..... 7

.2.1.4 Chemical composition of essential oils: ..... 7

.2.1.5 Role of essential oils ..... 8

.2.1.6 Methods of extracting the essential oils ..... 9

.2.1.6.1 Hydrodistillation ..... 9

2.1.6.2. Steam Entrainment ..... 9

2.1.6.3. Cold extraction ..... 9

.2.1.6.4 Extraction by volatile solvents ..... 9

.2.1.6.5 Extraction by Enfleurage ..... 10

2.1.7. Biological Activities of Essential Oils ..... 10

.2.1.7.1 Antioxidant activity ..... 10

.2.1.7.2 Antibacterial activity ..... 10
.2.1.7.3 Antifungal activity ..... 11
2.1.7.4. Antiparasitic activity ..... 11
2.1.7.5. Antiseptic Activity ..... 11

**Chapter 03: Materials and methods**

3.1.Plant material ..... **12**
3.2.The extraction of essential oils ..... **14**
3.3. The antifungal activity assay ..... **20**
3.3.1. Article of Mehani *et al*.,2015 ..... 20
3.3.2. Article of Tabet Zatla *et al*., 2017 ..... 21
3.3.3. Article of Ksouri *et al*., 2017 ..... 21
3.3.4. Article of Bouayad Alam *et al*.,2013 ..... 22
3.3.5. Article of Elhouiti *et al*., 2022 ..... 22
3.3.6. Article of Belmekki *et al*., 2013 ..... 23
3.3.7. Article of Dob *et al*., 2006 ..... 23
3.3.8. Article of Mazari *et al*.,2010 ..... 24
3.3.9. Article of Yakhlef *et al*., 2020 ..... 24
3.3.10. Article of Kacem *et al*.,2016 ..... 26
3.3.11. Article of Benomari *et al*., 2017 ..... 26
3.3.12. Article of Abi-Ayad *et al*., 2013 ..... 27
3.3.13. Article of Haoui *et al*., 2016 ..... 28
3.3.14. Article of Tolba *et al*., 2015 ..... 28
3.3.15. Article of Houicher *et al*., 2015 ..... 29

**Chapter 04: results and discussion**

4.1.The antifungal activity ..... **33**
4.1.1. Article of Mehani *et al*., 2015 ..... 33
4.1.2. Article of Tabet Zatla *et al*., 2017 ..... 34
4.1.3. Article of Ksouri *et al*., 2017 ..... 35
4.1.4. Article of Bouayad Alam *et al*., 2013 ..... 36
4.1.5. Article of Elhouiti *et al*., 2022 ..... 37
4.1.6. Article of Belmekki *et al*., 2013 ..... 38
4.1.7. Article of Dob *et al*., 2006 ..... 38
4.1.8. Article of Mazari *et al*.,2010 ..... 38

4.1.9. Article of Mazari *et al*., 2010 ..... 39
4.1.10. Article of Kacem *et al*., 2016 ..... 39
4.1.11. Article of Benomari and al., 2017 ..... 40
4.1.12. Article of Abi-Ayad and al., 2013 ..... 40
4.1.13. Article of Haoui and al.,2016 ..... 41
4.1.14. Article of Tolba *et al*., 2015 ..... 41
4.1.15. Article of Houicher *et al*., 2015 ..... 41
**Conclusion ..... 43**
**References list**

**Abstract**

## List of tables

**Table 1.** Plant species, region and date of harvesting……..……12

**Table 2.** Plant parts, methods used and the details of getting the essential oils……...15

**Table 3.** The most selected fungus and the antifungal assays…….……..30

## List of figures

**Figure 1.** Antifungal activity of *Mentha pepirita* on A: *Fusarium sporotrichioides*, B: *Fusarium graminearum* and C: *Fusarium langsethiae*……...……34

**Figure 2.** Preventive effect of root essential oil (A) after seven days of storage at 24 ± 1°c, controls (B)……..…….36

## List of abbreviations

**°C:** degree Celsius

**µg:** microgram

**µl:** microliters

**CFU:** colony forming unit

**cm:** centimeter

**DMSO:** Dimethyl sulfoxide

**EO:** Essential Oil

***et al*.:** and others

**g:** gram

**h:** hour

**$IC_{50:}$** inhibitory concentration

**$K_{aff}$** : affinity constant

**L:** litre

**mg:** milligram

**MHA:** Mueller-Hinton agar

**MIC:** minimum inhibitory concentration

**mL:** milliliter

**Mm:** millimeter

**MOPS:** propane sulfonic (N-Morpholino)-3 acid

**NA:** nutrient agar

**NaCl:** Sodium chloride

**nm:** nanometer

**OD:** Optical Density

**PDA:** Potato Dextrose Agar

**PH:** potential of hydrogen

# Introduction

## Introduction

Fungal infections are one of the most deadly infections accounting in excess of 1.5 million deaths annually worldwide. The major reason that makes fungal infections more life threatening because they are been neglected by the society.

Though in last 20 years there are many developments in the diagnosis and treatment of fungal disease but still majority of population are devoid of the benefits of these developments. Among all the fungal diseases, infection of skin hold the $4^{th}$ position and it accounts for the majority of death also. (Mishra *et al*., 2020).

Fungal infections are caused by eukaryotic organisms, and it is therefore more difficult to ascertain their presence and apply the appropriate therapeutic treatment compared to bacterial infections.

For thousands of years humanity has used various plants found in its environment, to treat and cure all kinds of diseases, these plants represent a huge reservoir of potential compounds attributed to secondary metabolites that have the advantage of being a great diversity of chemical structure and they possess a very wide range of biological activities. However, the assessment of these activities remains a very interesting task that may be the interest of many studies.

Only at the end of the 18th century that drugs are part of a scientific logic. Initially, it was discovered that plants contained several components; the first pure medicinal substance derived from the plants was morphine, which was extracted from the poppy at the beginning of the 19th century. Currently, aromatic plants have a major advantage due to the gradual discovery of the applications of essential oils in their health care and their uses in other areas of economic interest.

At present, approximately 25-30% of all the drugs available for the treatment of diseases are derived from natural products (plants, animals, bacteria and fungi).Today, many works, conducted in the field of ethno pharmacology, show that the plants used in traditional medicine and have been tested, are often effective and almost all free of toxic plants. The enhancement of natural resources is a concern that is becoming increasingly important in many countries as herbal medicines have more advantages compared to synthetic drugs. The African continent has a rich biodiversity among plants in the world, with a very high number of plants used as herbs, such as natural foods and for therapeutic purposes. Many different

natural substances have been identified and many of them are used in traditional medicine for treatment of diseases.

The cell wall of fungi may be considered as the prime target for selectively toxic antifungal agents because of its chitin structure, which is absent in human cells. Chemical treatments are largely effective, but resistant strains and intrinsically resistant species can be developed. The onset and severity of the fungal infection depends on the inoculum charge, the host's immunological state and resistance.

This work consists on a review of 15 research articles about the antifungal activity of essential oils, all of the chosen articles studied plants belonging to the Algerian flora. The material, methods, results and discussions are developed and compared.

# Theoretical framework

# Chapter01
# Fungi

# Chapter 01 Fungi

## 1.1. Fungi

### 1.1.1. Definition

Fungi are uni- or multicellular eukaryotic organisms with a filamentous appearance (Tabuc, 2007), immobile, some are microscopic (microfungi), others visible (macromycetes). Their wall is composed of chitin not cellulose as they are devoid of pigments and therefore unable to carry out photosynthesis. They are ubiquitous heterotrophic organisms whose carbon nutrition is dependent on the presence of preformed organic matter. It is the thallus or the mycelial filament that provides nutrition; this is done by absorption while first releasing hydrolytic enzymes into the external environment. Their vegetative apparatus is a thallus so they are thallophytes (Semal *et al*., 1993). They are classified into two main categories: the unicellular yeast form and the multicellular mycelial form made up of hyphae.

### 1.1.2. Classification

The general classification of fungi is based on the characteristics of the thallus (naked plasmodium or filament, septated or not; possible presence of naked flagellated cells), as well as on the modalities of their sexual reproduction (Semal *et al*., 1993). This fungal classification describes four main phylum (branches): chytridiomycetes, zygomycetes, ascomycetes and basidiomycetes, characterized by the nature of their spores. A fifth phylum considered artificial, the Deuteromycetes (Encarta, 2005).

#### 1.1.2.1. Chytridiomycetes

These are the oldest fungi (James *et al*., 2000). They are the only fungi to possess uniflagellate spores; Zoospore, (Jennings et Lysek, 1996). The presence of flagellated spores seems to restrict these organisms to aquatic environments and moist soils (James *et al*., 2000). The organisms of this phylum are often microscopic but can also produce mycelium. Most chytrids are saprotrophic, aerobic or anaerobic; they are capable to degrade a large number of substrates (Powell, 1993; Shearer *et al*., 2007).

#### 1.1.2.2. Zygomycetes

Zygomycetes are an ancient group of fungi that diverged after the Chytridiomycetes (James *et al*., 2000; Bar-Hen *et al*., 2008). Their reproduction is by an asexual way via spores forming inside a bag called sporocyst, as also they do the sexual reproduction one, which is by the union of sexual filaments whose extremities dilate, fuse to form a plurinucleated

zygospore. This set consists of two main orders: saprophytes, develop in the form of mycelium not partitioned like (*Rhizopus migricans*), or black mold of bread on the other hand, entomophtorales, whose sporocysts, usually serving themselves of spores, detach and are disseminated like conidia, these fungi are mainly insect parasites, fly killers (Encarta, 2005).

#### 1.1.2.3. Ascomycetes

Ascomycetes include $4_{50}00$ species described to date **(**Taylor *et al*., 2000). Also called sac fungi, include molds with septate hyphae and some yeasts. This group has a characteristic structure called ascus formed during sexual reproduction which encloses a definite number of ascospore. They also reproduce by asexual multiplication; they have a great economic importance because some species are able to degrade cellulosic substrates (Tabuc, 2007). They are the most important group of fungi, and the majority of species are microscopic (Bouchet *et al*., 1999).

#### 1.1.2.4. Basidiomycetes

They Are the most advanced fungi (Bouchet *et al*., 1999), characterized by basidiospores formed by sexual reproduction. These fungi have a partitioned mycelium, and their reproduction is sexual, occurring through an organ called basidium. This group includes the agents responsible for rust, coals and caries that develop on a very large number of plants (Fournier *et al*., 1983). Basidiomycetes rarely produce asexual spores, and their sexual spores mesh from mass-shaped basidium (Nicklin *et al*., 2000).

#### 1.1.2.5. Deuteromycetes

This group includes all fungi that do not produce ascospores or basidiospores and that multiply by conidia (Botton *et al*., 1990). Deuteromycetes are characterized by partitioned mycelium and vegetative reproduction by asexual spores, or by simple fragmentation of the mycelium (Boiron, 1996). These molds constitute the major part of the hyphals; they are classified according to the characteristics of the conidial organs and the mode of grouping of the hyphae. The Deuteromycetes group contain a large number of vegetal and foodstuffs contaminants including: *Trichoderma*, *Cephalosporium*, *Fusarium*, *Geotrichum*, this class regroup both *Penicillium* and *Aspergillus* (Frazier, 1967; Punt *et al*., 2002).

### 1.1.3. Role of fungi in nature

Fungi are great decomposers of organic matter. In ecology, saprophytic fungi participate in maintaining the ecological balance by releasing into the environment, from the matter they decompose, carbon and mineral salts; they develop on the most diverse materials as soon as the Humidity and temperature are favorable: harvests of cereals, fruits, vegetables, fabrics, leather, residential wood, books, even certain plastics, etc.

They also provide great services in the fields of health and agri-food. They produce enzymes, antibiotics like penicillin, and drugs like cyclosporine which are used to prevent organ transplant rejection. In biotechnology, fungi such as *Ashbya gossypii*, are exploited in the production of vitamins A, B or D (Santos *et al*., 2005).

In the agri-food industry, some mushrooms are used in cheese and pastry (Piskur *et al*., 2006). Like brewer's yeast, *Saccharomyces cerevisae*, which transforms sugar into alcohol and releases bubbles of carbon dioxide which cause the dough to rise, some *Penicillium* are used to make blue, Roquefort, Brie and Camembert cheeses.

In agriculture, fungi such as *Beauveria bassiana* are used in biological control. This fungus can be used to fight against the Colorado beetle in potato crops or against a caterpillar responsible for the corn borer.

By directly attacking living organisms, parasitic fungi are the cause of many diseases of humans, animals and plants. *Ceratocystis ulmi* invaded North America after World War I and virtually wiped out the American elm *Ulmus americana*. The ergot of rye, *Claviceps purpurea*, which parasitizes cereals, produces a molecule very close to lysergic acid. It is a powerful vasoconstrictor at the origin of Saint Anthony's fire, or Burning sickness, a disease that decimated thousands of people in the middle Ages: after consuming wheat parasitized by this fungus, the extremities become gangrene and fall off. This mushroom also contains a substance that treats high blood pressure.

Several fungi produce biological active compounds, including mycotoxins which are natural contaminants of the food chain, they are gaining increasing attention worldwide, due to the significant economic losses that are linked to their effects on the public health, animal productivity and trade (Castegnaro and Pfohl-Leszkowicz, 2002; Wu, 2006; Morgavi and Riley, 2007). They are secondary metabolites produced by molds belonging mainly to the genera *Aspergillus*, *Penicillium* and *Fusarium*. The latter are able to grow on a wide range of foodstuffs before, during and after harvest (Kabak *et al*., 2006; Reboux, 2006).

Several fungi are pathogenic, species of *Aspergillus*, in particular *Aspergillus flavus*, produce aflatoxins, toxins of the liver which grow in or on the seeds of peanuts. Consumption of these contaminated seeds leads to recurrent hepatic aspergillosis in the Third World (Maslin, 2004).

# Chapter 02

# Essential oils

# Chapter 02 Essential oils

## 2.1. Essential Oil

### 2.1.1. Definition

Essential oils also called essences. They are mixtures of aromatic substances produced by many plants and present in the form of tiny droplets in the leaves, the skin of the fruits, the resin, the branches and the woods. They are present in small quantities compared to the mass of the plant: they are fragrant and very volatile, that is to say, they evaporate quickly in the air (Bekhechi and Abdelouahid, 2014).

### 2.1.2. Location and Place of Synthesis

Essential oils exist almost only in higher plants: there are $17,_{50}0$ aromatic species. The genera capable of producing the constituents that make up essential oils are divided into a limited number of families, such as Myrtaceae, Rutaceae, Lamiaceae, Apiaceae, Cupressaceae, Poaceae and Piperaceae (Bruneton, 1999).

They are formed in the cytoplasm of variable secretory cells depending on the plant organ considered. Then, they generally accumulate in specialized glandular cells covered with a cuticle. Then, they are stored in specialized histological structures, often located on or near the surface of the plant, named, essential oil cells.

### 2.1.3. Physical properties

Essential oils are generally liquid at the ambient temperature, volatile, with a very strong smell, colorless, pale, yellow or sometimes blue. Their density is<1 except for the EOs of clove (*Syzygium aromaticum*), Cinnamon (*Cinnamomum zeylanicum*) and Sassafras (*Sassafras albidum*). They are insoluble in water but soluble in solvents, oils and petroleum jelly; highly alterable, they oxidize on contact with air and light (Charpentier *et al*., 2008). The term oil is explained by the property of solubility in fats and their hydrophobic character. The term “essential” refers to the fragrance and to the more or less strong smell released by the plant (Teusher *et al*., 2005).

### 2.1.4. Chemical composition of essential oils

Essential oils typically contain many of constituents that are distinct in chemical structures. Each constituent has its characteristics (Tisserand et Young, 2014). According to (Moro-Buronzo, 2008) we can find:

**Esters** which have an anti-inflammatory and cell regeneration properties. Among the essential oils rich in esters, we find those of lavender and Roman chamomile.

**Phenols** have a stimulating action; they are antiseptic and bactericidal, thus protecting the body from contamination. Among the essential oils rich in phenols, we find those of thyme, oregano and cinnamon.

**Aldehydes** are anti-inflammatory. They give a citrus scent to certain oils .Aldehyde-rich essential oils include orange, clove, lemon balm, lemongrass, and cinnamon.

**Ketones** have healing properties and help to clear mucus. Among the essential oils rich in ketones, we find those of eucalyptus, rosemary and niaouli.

**Alcohols** are among the most beneficial molecules in essential oils due to their antiseptic, antiviral, and analgesic properties. They are also immunostimulants. Among the EOs rich in alcohols, we find those of mint, lavender and tea tree.

**Terpenes** are very common in all essential oils. Some have antiviral properties even at very low concentrations. Terpene-rich essential oils include pine, mint, lemon, cypress, juniper, and rosemary.

**Acids** present in small quantities, but they have a powerful action. They are anti-inflammatory and sedative. Acid-rich essential oils include those of geranium, neroli, and juniper.

**Sesquiterpenes** have antiviral properties. Among the essential oils rich in sesquiterpenes, there are in particular those of clove, juniper and chamomile.

### 2.1.5. Role of essential oils

Plants use essential oils to protect themselves against viruses. Others consider oils to be messengers between sorts of parasites and microbes (Willem, 2013); works have shown that monoterpenes and sesquiterpenes can play important roles in the relationship of plants with their environment. For example, 1,8-cineole and camphor inhibit the sprouting of infected organs or the growth of pathogens from these organs.

Many plants produce essential oils as secondary metabolites, but their exact role in plant life processes is unknown (Rai *et al.*, 2003).

There is a lot of speculation about the "role" of plant essential oils. Certainly several “useful” apparent effects have been described: protection against infectious microbial flora by

fungicidal and bactericidal properties and against herbivores by taste and adverse effects on the nervous system (Porter, 2001).

Some authors believe that the plant uses the oil to repel or attract insects to promote pollination. Others regard the oil as a moisture preserver for plants in desert climates (Belaiche, 1979). Some essential oils serve to defend plants against herbivores, insects and micro-organisms (Capo *et al*., 1990).

### 2.1.6. Methods of extracting the essential oils

#### 2.1.6.1. Hydrodistillation

Hydrodistilation (water distillation) is the simplest method formerly used. The plant material is immersed directly in a still filled with water placed on a heat source. Everything is then brought to the boil, the heterogeneous vapors are condensed in a condenser and the essential oil separates from the hydrosol by simple difference in density. The essential oil being lighter than the water floats above the hydrolat (LUCCHESI, 2005).

#### 2.1.6.2. Steam Entrainment

The plant material is subjected to the action of a vapor current. Saturated vapors in volatile compounds are condensed then decanted before being separated into a phase aqueous and an organic phase (EO). The absence of direct contact between water and matter plant, then between water and aromatic molecules, avoids certain degradation phenomena such as hydrolysis (Bruneton, 1999).

#### 2.1.6.3. Cold extraction

Citrus fruit essential oils are fragile products due to their composition of terpenes and aldehydes. This is why, specifically for this category of raw material, a process totally different from a classic distillation is used, which is cold extraction (LUCCHESI, 2005**)**

The principle of this technique is based on breaking the walls of the oil bags contained in the rind of the fruits; this essence is then carried away by a current of cold water. The emulsion of gasoline and water, isolated by decantation or centrifugation (Ferhat *et al*., 2007).

#### 2.1.6.4. Extraction by volatile solvents

This method is used for plant organs with a relatively low concentration of essence or for essences that cannot be extracted by distillation. It is based on the power of certain organic solvents to dissolve the components of essential oils.

In this process, an exhaustion of the plants is carried out using a volatile solvent whose evaporation leaves a waxy, very colored and very aromatic residue called "concrete". The treatment of this concrete with absolute alcohol leads to the "absolute" (Duraffourd *et al*., 1990).

#### 2.1.6.5. Extraction by Enfleurage

It is a complex method; it is no longer used except for flowers. These are spread delicately on greasy plates which will absorb all the perfume. The fatty substances will then be exhausted by a solvent. Once the aroma of the flowers has been absorbed, the flowers are replaced by other fresh ones, and this until the fatty substance is saturated. After 24 hours, the fatty substance and the EO are separated (Moro-Buronzo, 2008).

### 2.1.7. Biological Activities of Essential Oils

#### 2.1.7.1. Antioxidant activity

Antioxidants are substances capable of protecting the body against the effects of oxidative stress. There are three types of antioxidants: enzymatic antioxidants, repair enzymes, and non-enzymatic antioxidants (Beirao and Gil, 2006). Natural substances including essential oils are classified as non-enzymatic antioxidants .Some studies have reported that certain EOs are more effective than synthetic antioxidants (Hussain *et al*., 2010).The antioxidant effects of EOs and plant extracts are mainly due to the presence of hydroxyl groups in their chemical structure (Hussain, 2009).

#### 2.1.7.2. Antibacterial activity

Due to the variability in the amounts and profiles of EO components, it is likely that their antimicrobial activity is not attributable to a single mechanism, but at several sites of action at the cellular level (Carson *et al*., 2002).

In general, a variety of toxic actions of EOs on bacteria have been observed, such as disruption of the cytoplasmic membrane, disruption of the proton motive force, electron leakage and coagulation of the protein content of cells (Davidson, 1997).

Nevertheless, certain low molecular weight phenolic compounds such as thymol and carvacrol can adhere to these bacteria by binding to parietal proteins and lipopolysaccharides thanks to their functional groups and thus reach the more vulnerable inner membrane (Dorman and Deans, 2000). (Defoe *et al*., 2003) had studied the chemical composition of essential oil *Thymus spinulosus* and carried out biological tests on its antibacterial activity

against strains of bacteria, the results showed that mono terpenes (thymol) has an inhibitory property of growth.

#### 2.1.7.3. Antifungal activity

In the phytosanitary and agro-food field, EOs or their active compounds could also be used as protective agents against phytopathogenic fungi and microorganisms invading foodstuffs (Lis-Balchin, 2003).

Essential oils act on a broad spectrum of mold and yeast by inhibiting yeast growth and spore germination, elongation of the mycelium, sporulation and toxin production in molds.

The antifungal power is attributed to the presence of certain chemical functions in the composition of essential oils. The antifungal action of these compounds is due to an increase in the permeability of the plasma membrane followed by a rupture of the latter leading to leakage of the cytoplasmic content and therefore the death of the yeast (Cox *et al*., 2000).

#### 2.1.7.4. Antiparasitic activity

Aromatic molecules possessing phenols have a powerful action against parasites. Linalool thyme and Mountain savory are excellent antiparasitic essential oils (Willem, 2002).

#### 2.1.7.5. Antiseptic Activity

Antiseptic and disinfectant properties are often found in essential oils possessing aldehyde or terpene functions such as *Eucalyptus radiata* essential oil (Willem, 2002).

# Experimental part

# Chapter 03

# Material and methods

# Chapter 03 Material and methods

This part presents all of the chosen plants and methods for the extraction of the essential oils, also the fungal strains tested by each article of the 15 ones.

## 3.1. Plant material

For each one of the mentioned articles, the different plants have been chosen for the extraction of the essential oils, the harvesting time and the region are listed in table 1

**Table 1:** plant species, region and date of harvesting

| articles | Plant species | region and date of Harvesting |
|---|---|---|
| **Mehani *et al*., 2015** | *Mentha pepirita* | Ain El Beida province of Ouargla in the south of Algeria in January 2015 |
| **Tabet Zatla *et al*., 2017** | *Daucus carota* subsp. *Sativus* | Mostaganem region, Northwest of Algeria in March 2014 |
| **Ksouri *et al*., 2017** | - *Origanum floribundum* Munby<br>- *Thymus ciliatus* Desf<br>- *Rosmarinus officinalis*L. | Two plants were collected from two places in Guelma region (East Algeria) in June 2010<br>- *O. floribundum* Munby harvested from Djebel Haouara<br>- *T. ciliatus* Desf harvested from Satha Ouled Sassi<br>Another plant was collected from Chain of Gora in Tebessa region(East Algeria) in January, 2010 which is *R. officinalis*. |
| **Alam *et al*., 2013** | - *Daucus crinitus*<br>- *Thymus capitatus*<br>- *Tetraclinis articulata* | Three pants were collected: *D. crinitus was* collected in Bensekrane forest area (Tlemcen Province) at the flwering stage in June 2011<br>- *T. capitatus*was collected from Beni snous in Tlemcen city at the flowering stage<br>- *T. articulata* was collected from Oujlida |

| | | |
|---|---|---|
| | | region, Tlemcen Province |
| **Elhouiti *et al*., 2022** | Four species of *Lamiaceae*: *Mentha piperita*, *Mentha pulegium*, *Thymus vulgaris* and *Thymus algeriensis* Boiss. and Reut.<br>Two other species of *Asteraceae*:<br>*-Artemisia herba-alba* Asso<br>*-Artemisia campestris*. | The species were collected from the region of ElHadjeub (Laghouat) and ElGhicha in the south of Djebel Amour in the Algerian hautes plaines in June 2017 |
| **Belmekki *et al*., 2013** | - *Teucrium polium* | The plant was collected in a full flowering situation at Beni-snous in the province of Tlemcen (Northwestern Algeria) in April, 2007. |
| **Dob *et al*., 2006.** | - *Thymus fontanesii* | Djelfa city, Algeria (300 Km south of Algiers) in May 2003 |
| **Mazari *et al*., 2010.** | - *Juniperus phoenicea*<br>- *Cupressus sempervirens* | - *J. phoenicea* was collected from Sidi Safi-Tlemcen (Algeria), in 21-11-2007<br>- *C. sempervirens* was collected from Maghnia-Tlemcen (Algeria), in 13-11-2007 |
| **Yakhlef *et al*., 2020** | - *Mentha rotundifolia* | The chosen plant was collected in its natural habitat in the Chenaoura locality in the commune of T'Kout, located in the Aures Massif 95 km south-east of the state of Batna (Algeria) during August September 2015 |
| **Kacem *et al*., 2016** | - *Genista quadriflora* | Was collected during the flowering stage near BouSaada, Algeria in June 2010. |
| **Benomari *et al*., 2017** | 11 location areas of Mentha were collected, 4 locations from *Mentha rotundifulia* (MRO1–MRO4), 2 locations from *Mentha* | Many samples were brought from western Algeria and collected at the flowering stage from 11 location areas of *Mentha*, during the period from July 2014 to September 2014 |

| | | |
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| | *spicata* (MSP1, MSP2), 3 locations from *Mentha pulegium* (MPU1-MPU3), and 2 locations from *Mentha piperita* (MPI1, MPI2) | |
| **Abi-Ayad *et al*., 2013** | - *Tetraclinis articulate* | the forest of Ghazaouet (Tlemcen city, Algeria), in April, November 2008 |
| **Haoui *et al*., 2016** | - *Inula viscosa.* | collected from trees growing in Sidi Rzine village (10 km far from Algiers), during December 2009 and June 2010 |
| **Tolba *et al*., 2015.** | - *Eucalyptus citriodora* | collected from the National Institute of Agronomy (El Harrach located 15 km of Algiers), in September 2014. |
| **Houicher *et al*., 2015.** | - *Artemisia campestris* | Collected during the flowering stage from Oued Moura, Laghouat region of Algeria. |

### 3.2. The extraction of essential oils

The used parts of the plants and extraction method of the essential oils are all shown in table 2

**Table 2.** Plant parts, methods used and the details of getting the Essential oils

| Articles | The used parts | Method used to get the EOs | Details of getting the EOs |
|---|---|---|---|
| **Article of Mehani *et al*., 2015** | The plant leaves | hydrodistillation | After the mint was harvested, The plant leaves were then dried in the shade in a dry and ventilated area protect from light. The extraction of essential oils was carried out by hydro distillation using Clevenger type apparatus. |

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| **Article of Tabet Zatla *et al*., 2017.** | The roots and total aerial parts | steam distillation | After roots and total aerial parts of *D. carota* subsp. *Sativus* were harvested, The essential oils were obtained by steam distillation for 5 h using Clevenger-type apparatus. The yields of the oils were, respectively, 0.2% from roots and 0.03% from the aerial parts. |
| **Article of Ksouri *et al*., 2017.** | the aerial parts of the three plants | Hydro distillation | After having all of the plant samples collected, Only the aerial parts of the three plants were used, they got air dried, then well conserved. Essential oils were extracted using Clevenger apparatus. The obtained essential oils were stored properly at 4◦C in tightly sealed dark-glass bottles. |
| **Article of Alam *et al*., 2013.** | Aerial parts | hydrodistillation | Since the aerial parts were collected, the *D. crinitus* oil yield was 0.37 % (w/w), *T. capitatus* yielded 0.52 % (w/w) and *T. articulata* yielded 0.31 % (w/w) The plant species were stored at -18 °C after harvest. A portion ($5_{50}$-600 g) of material from each plant species was subjected to a Clevenger-type apparatus according to the European Pharmacopoeia (EUROPEAN PHARMACOPOEIA, 2004). The essential oils were dried over anhydrous sodium sulfate and, after filtration, stored in sterilized amber vials at 4 °C until it was used. |
| **Article of** | the aerial parts | hydro-distillation | After getting the selected plant parts, |

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| **Elhouiti *et al*., 2022.** | | | they were dried at room temperature, away from light and humidity. Essential oils were obtained by hydro-distillation during 2.5 to 3 h, using a Clevenger type apparatus (Clevenger, 1928). The obtained oil was treated by anhydrous sodium sulfate, filtrated then stored at 4°C, until analysis. |
| **Article of Belmekki *et al*., 2013.** | Aerial part | steam-distillation | The essential oil was isolated by steam-distillation from the dried aerial parts of *T. polium* during 3 h. The sample oil was dried over anhydrous sodium sulphate and stored at low temperature before analysis. |
| **Article of Dob *et al*., 2006.** | The aerial parts (stems + leaves) | Hydrodistillation | The shade-dried and finely powdered aerial parts of the plant were exhaustively extracted by hydrodistillation for 3 h using a Clevenger- type apparatus with a water cooled receiver in order to reduce hydrodistillation overheating artifacts. The oil was extracted from the distillate with diethyl ether and then dried over anhydrous sodium sulfate. After filtration, the solvent was removed by distillation under reduced pressure in a rotary evaporator.<br>Oil was obtained in a yield of 0.9 % based on dried weight of sample. The oil was stored in a sealed glass vial in |

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| | | | the dark at 4 °C until the bioassay test. |
| **Article of Mazari *et al*., 2010.** | Leaves | Steam distillation | The dried plant samples (leaves) were subjected to steam distillation for 3 hours. Samples oils were dried over anhydrous sodium sulphate and stored at low temperature before the bioassay test. |
| **Article of Yakhlef *et al*., 2020.** | stems + leaves | Hydrodistillation | The essential oil was obtained by hydrodistillation of 100 g of plant material using a simple assembly of hydrodistillation for 2.5 h. The distillate was saturated with NaCl and then extracted using a collector solvent (10 mL, diethyl ether), as indicated in the literature (Djebaili *et al*., 2013; Joshi *et al*., 2013). The resulting organic phase was dried on anhydrous sodium sulfate. Diethyl ether was removed carefully under reduced pressure in a rotary evaporator at 35 ◦C. Finally, the pure oil was kept at 4 ◦C, in the dark inside well-sealed opaque vials, until the moment of analysis. The oil yield was calculated relative to the dry matter (three replicates were performed). |
| **Article of Kacem *et al*, 2016.** | aerial parts composed of stalks, leaves and flowers | Hydrodistillation | The plant material (100 g) was cut in small pieces furthermore subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The yield (%) was calculated as volume (ml) of |

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| | | | extracted essential oil per 100 g of plant material. Then, the essential oil was stored in hermetically sealed dark-glass and stored in freezer at 4 ◦C until the analysis. |
| **Article of Benomari *et al*., 2017.** | the fresh aerial parts of all stations | Hydro distillation | From each location that was mentioned in the table1 many samples were collected to poll collective oils from the same species. Essential oils were obtained from the fresh aerial parts of all stations by hydrodistillation for 4 h using a Clevenger-type apparatus according to the European Pharmacopoeia (Council of Europe, 1997) and yielded (w/w) 0.78–0.96% from *M. rotundifulia*, 0.$_{50}$–0.56% from *M. spicata*, 0.70–0.75% from *M. pulegium*, and 0.67–0.72% from *M. piperita*. |
| **Article of Abi-Ayad *et al*., 2013.** | The terminal branches (leaves) | Hydro distillation | Hydrodistillation of the dried plant samples ($_{50}$0 g of plant material in boiling water) were performed in a Clevenger type apparatus for 3 h and half. The oil obtained was light yellow, liquid at room temperature and its odor was agreeable. After its isolation, the essential oil was collected and stored in steeled glass vials in refrigerator at 4 ◦C. |
| **Article of Haoui *et al*., 2016.** | Leaves | steam distillation process | The essential oil was extracted by steam distillation process. The steam is generated using a boiler and enters |

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| | | | a column containing the plant material (16 g foliage). The passage of steam through the plant material produces a mixture "water-oil" which goes through a condenser to be collected in a graduated glass recipient. The process was continued for 5 h from the first distillate drop. The experiment was repeated three times. The collected oil samples were water-separated, measured, dried over anhydrous sodium sulphate and stored at 4°C until use. The compound yield is calculated by the following formula: Compound yield (%) = Extraction yield (%) x Compound content (%) / 100 |
| **Article of Tolba *et al*., 2015.** | Leaves | steam distillation | *E. citriodora* essential oil was isolated by process of conventional steam distillation apparatus. The vapor produced by the steam generator crosses the plant, charged with essential oil and then passes through the condenser to a receiving Florentine flask. The obtained essential oil was dried over anhydrous sodium sulphate and, after filtration, stored at 4 ◦C until tested. |
| **Article of Houicher *et al*., 2015.** | The aerial parts | steam distillation | The essential oil was isolated by hydrodistillation for 4 h from the dried aerial parts using a Clevenger-type apparatus, according to the method recommended in the British |

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| | | | Pharmacopoeia. |

❖ As a recapitulation :

Lot of plant species were selected for the extraction of the EOs by each one of the mentioned articles, that were also harvested in a region and time totally different.

Different plant parts were used by the mentioned articles for the extraction of the EOs where,

The Article of Ksouri *et al*., 2017, Article of Alam *et al*., 2013, Article of Elhouiti *et al*., 2022, Article of Belmekki *et al*., 2013, Article of Benomari *et al*., 2017 and the Article of Houicher *et al*., 2015, have used only the aerial parts of the selected plants

All the article of Mehani *et al*., 2015, Mazari *et al*., 2010, Abi-Ayad *et al*., 2013, Tolba *et al*., 2015 and the Article of Haoui *et al*., 2016, used the leaves of the plant for the extraction of the oils.

Another article has used the aerial parts composed of stalks, leaves and flowers which is the Article of Kacem *et al*., 2016, though other two articles have used aerial parts but composed only of stems and leaves of the plant which are the Article of Dob *et al*., 2006 and the Article of Yakhlef *et al*., 2020.

Only in the article of Tabet Zatla *et al*., 2017 roots and total aerial part were used for the extraction of the essential oil.

For the EOs extraction methods of each article, only two methods had been used which were both steam and hydrodistillation.

Concerning the used apparatus for the extraction, only the Clevenger type apparatus was used in common for all the aforementioned articles researches.

### 3.3. The antifungal activity assay

Each article have selected different fungal strains to test the effect of the extracted essential oils

#### 3.3.1. Article of Mehani *et al*., 2015

In this article the selected method for the antifungal assay was direct contact. Three species of *Fusarium* genus (*Fusarium sporotrichioides*, *Fusarium graminearum* and *Fusarium langsethiae* ) have been taken for the examination of the *Mentha piperita* effect on

them. Different concentrations were taken from the *Mentha pepirita* EO ($_{50}$, 10, 5, 2.5, 1.25, µl) and adjusted to 20 ml PDA then stirred for 5 minutes till get the medium PDA and the EO well homogenized.

### 3.3.2. Article of Tabet Zatla *et al*., 2017

Three species of fungi *Aspergillus niger, Penicillium expansum* and *Botrytis cinerea* were taken from a strawberry greenhouse by harvesting from diseased fruits and maintained in potato dextrose agar (PDA, Sigma–Aldrich, USA) in a growth chamber at 23°C for 14 days in the darkness. Each isolate was purified by a single spore isolation technique and identified based on cultural and morphological characteristics, spore were obtained from the surface of the agar and suspended in 5 mL of sterile distilled water containing 0.1% v/v Tween 80. Spore suspensions were filtered through four layers of sterile cheesecloth to remove mycelial fragments. The number of spores was calculated with an automated cell counter (Bio-Rad's TC20), Cellometer Vision and the spore concentration was adjusted to $1 \times 10^6$ spores/mL with the aid of a haemocytometer prior to use.

The Antifungal activity of the EOs was tested against the aforementioned strains The method used was a modification of Tian *et al*., which was based on a method published by Singh *et al*. Aliquots of extracts dissolved separately in 0.5 ml of 10% (v/v) DMSO were pipetted aseptically to 9 cm glass Petri dishes containing 9.5 ml of molten PDA to procure the requisite concentrations of essential oils (30 and 70 mL/L for roots and 100 and 400 mL/L for aerial parts) Control plates (without essential oils) were inoculated following the same procedure. A 9 mm fungal disc of mycelial material, cut from the periphery of a five-day-old culture with a sterile cork borer, was inoculated aseptically to the centre of the prepared treatment and control Petri dishes. The plates were sealed with polyethylene film and incubated at a temperature of 27°C. The efficacy of the treatment was evaluated each day for 7 days by measuring the average of two perpendicular diameters of the colony. All tests were performed in triplicate. Percentage inhibition of the radial growth of the tested fungi by the oil, compared with the control was calculated at day seven, using the following formula:

I% = {DC-DT/DC}*100, where DC and DT are average diameters of fungal colony of control and treatment, respectively.

### 3.3.3. Article of Ksouri *et al*., 2017

In this study it was often used the broth macrodilution in tubes described by Giordani *et al*., for the evaluation of the antifungal activity. A stock solution for each essential oil was

prepared by adding 0.5 mL of sterile saline water to 0.5 mL of essential oil. Ten dilutions were prepared from the stock solution in sterile test tubes: 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512 and 1/1024. To prepare the fungi sample, *Candida albicans* was chosen for the test, where, Ten milliliters of Sabouraud gentamycin broth (0.1 g/l) was inoculated with a young culture of *C. albicans* (24 h) to prepare a suspension of 0.5 McFarland. 0.9 mL of the ICM (inoculated culture media) suspension was mixed with 100 mL of each oil dilution. The tubes were incubated in shaking water bath at 35 ◦C for 24 h. OD (Optical Density) reading is done at 560 nm against the culture medium. Data (measured OD) were subjected to a statistical analysis to determine the MIC at 80% and the affinity constant ($k_{aff}$) of essential oils for yeast. The graphic representation of fungal growth inhibition according to essential oil concentrations has a hyperbolic appearance with the equation: Y= ax/ (b + x)
a: the calculated maximum inhibition, b: antifungal agent concentration that induces an inhibition equals to the half of the calculated maximum inhibition.
The use of a software for adjustment (developed by Faculty of pharmacy, University of the Mediterranean) allows to determine the parameters a and b. Noting that MIC 80% is the minimum inhibitory concentration that prevents visible growth of the tested strains at a rate of 80% compared to the control (ICM).

### 3.3.4. Article of Alam *et al*., 2013

In this research the selected fungi for the EO antifungal activity that has been extracted from three plants were *Fusarium oxysporum*, *Alternaria solani*, *Aspergillus niger*, *Penicillium sp*1 and *Penicillium sp*2, were isolated from naturally decayed tomato after storage of several weeks. Those isolates were the most aggressive one in their collection and produced the largest lesions on inoculated fruit. A pure culture of those fungus were maintained on potato dextrose agar medium (PDA: potato 200, dextrose 20 g and agar 15 gL-1 in distilled water at 25 °C) in the presence of a quantity of lactic acid (25 %) to stop the growth of bacteria. The plates were incubated at 25 ± 2 °C for 8 days in darkness. The developing fungal colonies were purified and identified up to the species level by microscopic examination through the help of the following references (Barnettet Hunter, 2006).

### 3.3.5. Article of Elhouiti *et al*., 2022

In this research, they tested the antifungal activity on five strains; INRA 349 of *F. graminearum* from CBS collection 185.32 (Centraalbureau voor Schimmelcultures, Netherlands), BD17 of *Fusarium culmorum* from the collection of Touati-Hattab Sihem (Touati-Hattab *et al*., 2016), *Fusarium oxysporum* f. *sp*. *lycopersici* (FOL) from National

Institute of Agronomy (El Harrach), *Fusarium oxysporum* f. *sp*. *Albedinis* (FOA) from Regional Plant Protection Station (SRPV Ghardaïa) and *Fusarium oxysporum* f. *sp*. *pisi* (FOP) obtained from Mycology laboratory of agronomy department (University of Blida). After fungus done selected, the followed method to evaluate the EO effect on mycelial growth and the determination of inhibition rates in solid culture medium were described in a previous study (Elhouiti *et al*., 2017). In brief, 1/5th–1/200th dilutions of the EOs were prepared in an agar solution (2%). In tubes, each containing 13.5 ml of sterile PDA medium (45°C), 1.5 ml of each EO dilution was added to obtain final concentrations ranging from 0.5 to 20 µl/ml. The effect of the EOs on mycelial development was evaluated by calculating the inhibition percentage of the .mycelium after 7 days of incubation at 25 ± 2°C.

### 3.3.6. Article of Belmekki *et al*., 2013

The tests were carried out by insemination, with mycelia fragments of 6 mm in diameter (3 at 5 days hold), in Petri dishes containing PDA (Potato dextrose agar) (Fandohan *et al*., 2004). The oil was tested at the different concentrations of 0.25; 0.5; 1.25; 2.5; 5 and 10 µl/ml. These concentrations were obtained by mixing 0.5, 10, 25, $_{50}$, 100 and 200 µl of essential oil with 20 ml of melted sterile PDA respectively. A disc (6 mm diameter) of the fungal species was cut from 1 week old cultures on PDA plates and then the mycelia surface of the disc was placed upside down on the center of a dish. Then, the plates were incubated in the dark at 25 ± 2°C. The extension diameter (mm) of hyphae from centers to the sides of the dishes was measured after 3 to 5 days. In addition, PDA plates treated with amphotericin B (200 µg/ml) were used as positive control. The Petri dishes containing 20 ml of PDA with no oil was inoculated to serve as the negative control. Fungal growth was evaluated by measuring the average colony diameters after 3 to 5 days (Salamci *et al*., 2007). The percentage of growth inhibition was calculated using the following equation: % Inhibition = (C –T)/ C x 100. Where C, is the average of four replicates of hyphal extension (mm) of controls and T, is the average of four replicates of hyphal extension (mm) of plates treated with essential oil solutions (Salamci *et al*., 2007).

### 3.3.7. Article of Dob *et al*., 2006

The EO antifungal activity in this research was evaluated on two yeasts (*Saccharomyces cerevisiae, Candida albicans*), and two filamentous fungi (*Mucor ramanianus* NRRL 6606, *Fusarium oxysporum sp albedinis*) where the fungal strains were obtained from the Microbiological Laboratory, Department of Biology, Ecole Normale Superieure, Algiers, Algeria. Cultures were maintained on nutrient agar (NA) medium. Diffusion method using

filter paper disk was used for the screening of oil antifungal activities. For this, organism suspension was prepared with sterile physiological water uniformly mixed with sterile liquid nutrient agar in Petri dishes. Sterile filter paper disks (9 mm) were soaked with 30 mL of oil and placed on the surface of NA medium. Petri dishes were placed at 4◦C for 24 h to allow the diffusion of the oil from disk to medium. After the incubation period (28◦C for 36–48 h) the inhibition halo diameters were measured using a ruler and expressed in millimeters.

### 3.3.8. Article of Mazari *et al*., 2010

In this research, there were two EOs extracted from two plants to evaluate individually their effects on 3 fungi (*Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus stolonifer*). The fungal strains were obtained from the Microbiology laboratory in Biology, Science Faculty of the Tlemcen University Algeria.

The antifungal activity of the EOs in this study was tested by using a direct contact assay (Fandohan *et al*., 2004) where; Five hundred microliters of the oil were mixed with 20 ml of the PDA (Potato dextrose agar) and were then put in the Petri dish (final concentration is 25 µl/ml). A disc (6 mm in diameter) of the fungal species was cut from (2 - 5)-days-old cultures on PDA plates and then the mycelia surface of the disc was placed upside down on the centre of a dish with fungal species in contact with growth medium on the dish. Then, the plates were incubated at 25 ± 2°C. PDA plates, without essential oils, were used as negative control. In addition, PDA plates treated with amphotericin B (200 µg/ml) were used as positive control. The diameters of growth of the hyphae were measured after 48 h and 7 days, respectively, for *Rhizopus stolonifer* and for the others strains. All the experiments were replicated three times. The percentage of growth inhibition by treatment (T) was calculated using the following of Ebbot (Motiejūnait- and Peičulyt, 2004): $T = [(Dk - D0) / D K] * 100$, Where: Dk and D0 are the average of three replicates of hyphal extension (mm) of controls and of plates treated with EOs.

### 3.3.9. Article of Yakhlef *et al*., 2020

This study depended in the evaluation of the EOs antifungal effect on taking a field pathogen: *Fusarium culmorum*. This mold (isolate F1; accession number: KP726896) was brought from the mycotheque of the laboratory of Cellular and Molecular Biology from the University of the Mentouri Brothers Constantine 1. The strain was isolated from cereal plants with characteristic symptoms of *Fusarium* wilt; namely unilateral leaf wilt and browning of

their margins. It has been identified based on its morphological, biochemical and molecular characters (Sebihi, 2016).

Two methods were applicated for the study of *Mentha rotundifolia* EO effect on fungus strains which were Dilution method in a solid medium and microatmosphere method.

For the dilution method in a solid medium, the antifungal assay was carried out by agar incorporation method (De Billerbeck *et al*., 2002; Angaman *et al*., 2018). Because of their hydrophobic nature, the EO was diluted in the in DMSO, six concentrations were selected 40, 20, 10, 5, 2.5 and 1.25 µL/mL; the solvent DMSO was used as a negative control. A volume of 2 mL of each concentration of extract was added to 18 mL of Potato Dextrose Agar medium (PDA) before solidification to give final concentrations: 4, 2, 1, 0.5, 0.25, and 0.125 µL/mL of agar. After homogenization, this mixture was poured into Petri dishes. The inoculation was carried out on the surface, in form of deposits of a mycelia fragment about 5–6 mm aged of 7-day old, taken at the level of the growth front and placed at the geometric center of the petri dish, on the solidified medium. The inoculated Petri dishes were sealed with adhesive film and incubated at 25 ± 2 ◦C for 7 days. A measurement of the diameters of different colonies was made at the end of incubation to calculate inhibition rates:

I (%) = [(Dc – Dt) / Dc] *100. Where, Dc: mycelia growth in the control plate; Dt: mycelia growth in the test plate.

Microatmosphere method, that help studying the effect of EO volatile fraction (Laghchimi *et al*., 2014). In this technique, a Petri dish (90 mm) containing 20 mL of PDA agar medium (20 mL of medium MH (Mueller-Hinton) offers 80 mL of air in each plate) was fist seeded with a 5−6 mm diameter fragment taken from the periphery of a fungal culture in the seven-day PDA medium; then a disk of Wattman paper soaked with a quantity of pure essential oil was deposited in the center of the lid of the Petri dish: 0 (control), 10, 20, 40, 80, and 160 µL/disk (equivalent to 0, 0.125, 0.25, 0.5, 1, and 2 µL/mL air). Immediately, the plates were sealed using parafilm to prevent evaporation of the EO, and then incubated in the dark, lid down, for seven days at 25 ± 2 ◦C. Each test was replicated twice. A control, devoid of EO, is prepared under the same conditions with a disc of paper impregnated with distilled water. The fungitoxicity, expressed as percentage inhibition of mycelium growth (I %), was calculated according to the formula: I (%) = [(Dc – Dt) / Dc] *100. Dc: mycelia growth in the control plate; Dt: mycelial growth in the test plate. The mentioned measurements were used to determine the minimum inhibitory concentration (MIC).Study of the nature of the fungitoxicity of the essential oil studied. The distinction between minimum fungistatic

concentration (MFC) and minimum fungicidal or lethal concentration (MLC) was determined by the transfer of mycelia fragments from petri dishes, where inhibition by the EO was complete in both techniques used, in a new PDA medium EO devoid. The essential oil is considered fungistatic if the growth of the fungus resumes again and is fungicidal or lethal if there is no growth.

### 3.3.10. Article of Kacem *et al*., 2016

In this research, the depended way to study the antifungal activity of the EO extracted from *G. quadriflora* was by the disk diffusion method (Ouhdouch *et al*., 2001). Four filamentous fungi obtained from the Laboratory of Microbial Engineering and Applications (University Constantine 1) *Aspergillus niger* CIP 1431, *Alternaria sp*., *Fusarium oxysporum* CIP 625.72, and *Trichochyton rubrum* CIP 2043.92 were used. Test strain suspension was prepared individually by culture growth in 20 ml Bennett's medium for 24 h at 30 ◦C. The suspension was adjusted to contain approximately $10^6$ colony-forming units (CFU)/ml. Typically, five hundred microliters of a prepared suspension of the microorganisms was spread with a sterile cotton swab on the surface of Mueller-Hinton agar (MHA) plates at 37 ◦C and allowed to dry for 10 min (Isu and Onyeagba, 2002). A stock solution of the essential oil was prepared by dissolving 20 mg in 1 ml of methanol. Sterile filter papers (5 mm in diameter Whatman disks) were impregnated with 20 µl of 20, 10, 5 mg/ml solutions. The Petri dishes with Sabouraud media were kept at 4 ◦C for 2 h to allow the diffusion of the oil, and then incubated at 30 ◦C for 72 h. The formation of a clear zone around the sample impregnated disk was used as an indicator of antifungal activity. Disk impregnated with the methanol was used as a negative control. Tests were performed in triplicate.

### 3.3.11. Article of Benomari *et al*., 2017

In this research the chosen fungi for doing the study were *Botrytis cinerea* and *Penicillium expansum* strains obtained from Institute Pasteur, Paris, France (IP 1854.89, batch 185489; IP 1405.82, batch $0_{50}$291 and IP $13_{50}$.82, batch 230992). *Monilinia laxa* and *Monilinia fructigena* strains obtained from Centraal bureau voor Schimmel cultures, Utrecht, Netherlands (CBS 127258, batch 127258 and CBS 101499, batch 101499). The fungal strains were grown on Sabouraud Glucose Agar (Sigma - Aldrich, Saint -Louis , Missouri, USA) and incubated at 23 °C in darkness

The essential oils were evaluated for fumigant antifungal activity based on their ability to inhibit mycelia growth. Six-millimeter diameter mycelila plugs of each fungal strain from a

7-day-old culture were placed into a Petri dish. Essential oils were introduced onto a 6-mm cellulose disc, placed on the agar-free lid of the Petri dish. A negative control (cellulose disc without essential oil) was performed in the same way. Petri dishes were then sealed with a parafilm and incubated at 23 °C in the dark. Mycelial radial growth was measured after 3 to 7 days of incubation, and the antifungal index was calculated with the formula as follows: Antifungal index(%) = (1- Dex /Dc)*100 where, Dex is the diameter of growth zone in the experimental plate (mm) and Dc is the diameter of growth zone in the control plate (mm). After a preliminary assay to determine antifungal index, Minimal Inhibitory Concentration (MIC) was determined for more active essential oils. In each treatment, the MIC was determined, with two replicates for preliminary assays and three replicates for the determination of MIC.

### 3.3.12. Article of Abi-Ayad *et al*., 2013

Three pathogenic fungal strains were selected for their study *Aspergillus flavus*, *Aspergillus niger*, *Fusarium spp* were obtained from the culture collection of the Tlemcen University (Laboratory of Mycology), and each one of their culture were maintained on potato dextrose agar (PDA) and stored at 4 °C.

The depended method to study the EO effect was a contact assay in vitro. For *Fusarium spp*. (De billerbeck *et al*., 2001; Tatsadijeu *et al*., 2009). Briefly, PDA plates were prepared using 9 cm diameter glass Petri dishes. Different concentrations of essential oil (10, 15 and 20 µL/ml) were prepared by adding appropriate quantity of essential oil to melted medium, followed by manual rotation to disperse the oil in the medium to obtain a final volume of 20 ml. A mycelia disc (6 mm diameter) of the *Fusarium spp*. was cut from 1 week-old culture on PDA plates. Then, the mycelia surface of the disc was inoculated at the center of a dish. For *A. flavus* and *A. niger*. An emulsion of essential oil was prepared in Agar to give a volume of 2 ml (Bourkhiss *et al*., 2007), this volume was added to 18 ml of PDA medium to obtain a final concentration of 5, 10 and 15µl/ml. The 20 ml of medium were poured into glass petri dishes (9 cm diameter). Each petri-dish was inoculated by a spot of 100 µL made of a suspension of about $10^7$spores/ ml in distilled water containing 0.01 % of Tween-80 to improve spore dispersion (Tantaoui-Dlaraki et Baroud,1992). The spores were collected from a five-day culture of *A. flavus* and *A. niger* on PDA medium at 25 ◦C. Control plates (without essential oil) were inoculated following the same procedure for each strain. Then the plates were incubated at 25 ◦C for 6–8 days. The extension diameter (mm) of hyphae from centers to the sides of dishes (colony diameter) was measured at 24 h intervals. The percentage of

mycelial growth was calculated using the following formula: (De Billerbeck *et al*., 2001; Tatsadijeu *et al*., 2009):inhibition% = [ (Gc-G**0**)/ Gc ] * 100 where, GC = mean diameter for control and GO = mean diameter for treated mycelium, and for All statistical analysis were performed using Origin Lab version 7.5 (Origin Lab Corporation, 2003).

### 3.3.13. Article of Haoui *et al*., 2016

Two species of fungi *Fusarium culmorum* and *Fusarium graminearum* were selected to test the *I. viscosa* essential oil effect on them. Then both of EO and fungi were tested; the agar dilution method used for fungi testing and the oil was diluted till reaching the concentrations 762, 1523 and 6092 μg/mL).

The tested oils were added to the (PDA) at a temperature of 40-45°C, and then poured into Petri dishes (10 cm diameter). The molds were inoculated as soon as the medium had solidified. Disk of the fungi mycelium (9 mm diameter) was placed at the center of each Petri dish and incubated at 25°C. The treatment efficacy was evaluated after 7 days by measuring the colonized fungus diameters. Each experiment was performed in triplicate. The inhibition percentage was calculated using the following equation:

Inhibition (%) = (Growth area in reference - Growth area in sample) / (Growth area in reference) x 100

### 3.3.14. Article of Tolba *et al*., 2015

In this article, there was taken four samples of fungi *Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum* to evaluate effect of the chosen EO which was procured from Pasteur Institute, Algiers, Algeria. Fungal pathogens were grown on (SDA) for 7—14 days at 27 8C, after which time spores were prepared by gently scraping the culture surfaces using a sterile glass after macerating in sterile saline (0.9%) solution. The final spore suspension was adjusted to $10^5$—$10^6$CFU/mL.

Concerning the antifungal study, there had been selected the disc diffusion method using SDA (Sabouraud Dextrose Agar) in Petri dishes (90 mm diameter). The sterile discs (6 mm diameter) were impregnated with 3 different concentrations (10, 20, 30 mL/ disc) of the oil and placed on the surface of the media previously inoculated with fungal suspension ($10^6$CFU/mL). The tested plates were incubated at 27 8◦C for 7 days and the inhibition diameters were measured. All tests were performed in triplicate.

- **Disc volatilization method**

This method describes the diffusion of the essential oil in vapor phase. Three different volumes (10, 20 and 30 mL per disc) of the essential oil were pipetted on the paper disc (diameter 6 mm) and placed on the inside surface of the upper lid of Petri dishes. The lids were immediately closed with the plates containing the solid media SDA previously inoculated with microbial suspension ($10^6$CFU/mL) and sealed with parafilm to prevent leakage of the EO vapor. Plates were inoculated at 27 ◦C during 7 days, and the diameter of the inhibition zone was measured. Tests were carried out in triplicate.

### 3.3.15. Article of Houicher *et al*., 2015

In this research, the antifungal activity was tested against 10 strains of fungi where, two strain types were brought from the ARS (Agricultural Research Service) culture collection (NRRL) **(***Aspergillus ochraceus NRRL 3174, Aspergillus flavus NRRL 3251),* four strain types brought from the Belgian Coordinated Collections of Micro-Organisms (*Fusarium graminearum* MUCL 53452, *Fusarium moniliforme* MUCL 53645, *Penicillium citrinum* MUCL 31475, *Penicillium expansum* MUCL 29192), and one strain type from the CBS cultures collections of micro organisms (*Aspergillus parasiticus* CBS 100926). The other strains (*Penicillium viridicatum*, *Aspergillus niger*, and *Fusarium culmorum*) were from the culture collection of the Department of Agriculture, Faculty of Science, Laghouat University-Algeria. Prior to antifungal susceptibility testing, each isolate was cultured on potato dextrose agar (PDA) for 7-14 days at 25°C to ensure rapid sporulation and purity.

For the study of the antifungal activity, a broth macrodilution method was used to determine the Minimal Inhibitory Concentrations (MIC) and Minimal Fungicidal Concentrations (MFC), according to the Clinical and Laboratory Standards Institute M38-A for filamentous fungi. For the assay, the essential oil was twofold diluted in dimethyl sulfoxide (DMSO) (Sigma 34943, USA), with concentrations ranging from 0.04 to 20 µl/ml. The final concentration of DMSO was ≤1%. Recent cultures of each strain were used to prepare the cell suspension adjusted to 0.4–5×104 colony-forming unit (CFU)/ml for filamentous fungi using a spectrophotometer (Jenway, 6405 UV/VIS, UK) at 530 nm. All tests were performed in RPMI-1640 medium (Sigma $R6_{50}4$, USA) buffered to a pH 7.0 with MOPS buffer (Sigma M3183, USA) at a concentration of 0.164 mol/l. After inoculation, the test tubes were incubated aerobically at 35°C for 48 h/72 h, including two control tubes per strain and then the MICs were determined. The MIC was defined as the lowest essential oil

concentration preventing any fungal growth as detected visually. After MIC reading, aliquots (20 µl) of broth from each negative tube were taken and cultured in Sabouraud dextrose agar (Eur-Pharm, 1024.00, Spain) plates in order to evaluate MFCs. The plates were then incubated for 72 h at 35°C. The MFC was defined as the lowest essential oil concentration showing either no growth or fewer than three colonies to obtain an approximately 99–99.5% killing activity. All experiments were performed in triplicate and repeated if the results differed.

In the goal of evaluating the antifungal effect of the essential oils, different methods and fungal strains were selected for doing the researches are summarized in the table 4

**Table 3:** The most selected fungus and the antifungal assays

| Articles | Fungal strains | Antifungal assay method |
|---|---|---|
| **Article of Mehani *et al*.,2015** | - *Fusarium sporotrichioides, Fusarium graminearum, Fusarium langsethiae* | - direct contact |
| **Article of Tabet Zatla *et al*., 2017** | - *Aspergillus niger, Penicillium expansum, Botrytis cinerea* | / |
| **Article of Ksouri *et al*., 2017** | - *Candida albicans* | - The broth macrodilution in tubes |
| **Article of Alam *et al*., 2013** | - *Fusarium oxysporum, Alternaria solani, Aspergillus niger, Penicillium sp*1, *Penicillium sp*2 | / |
| **Article of Elhouiti *et al*., 2022** | - *Fusarium graminearum, Fusarium culmorum, Fusarium oxysporum* sp. *Lycopersici, Fusarium oxysporum* sp. *Albedinis, Fusarium oxysporum* sp. *Pisi* | / |
| **Article of Belmekki *et al*., 2013** | - *Aspergillus flavus, Fusarium oxysporum ,Rhizopus stolonifer* | Insemination |
| **Article of Dob *et al*., 2006** | - *Saccharomycescerevisiae, Candida albicans, Mucor ramanianus* NRRL | - Diffusion method |

| | | |
|---|---|---|
| | 6606, *Fusarium oxysporum* sp. *albedinis*). | using filter paper disk |
| **Article of Mazari *et al*.,2010** | - *Aspergillus flavus, Fusarium oxysporum ,Rhizopus stolonifer* | - direct contact assay |
| **Article of Yakhlef *et al*., 2020** | - *Fusarium culmorum* | two methods:<br>- Dilution method in a solid medium<br>- microatmosphere method. |
| **Article of Kacem *et al*.,2016** | - *Aspergillus niger*CIP 1431, *Alternaria sp*., *Fusarium oxysporum* CIP 625.72, *Trichochyton rubrum* CIP 2043.92 | - disk diffusion method |
| **Article of Benomari *et al*.,2017** | - *Botrytis cinerea, Penicillium expansum., Monilinia laxa, Monilinia fructigena* | / |
| **Article of Abi-Ayad *et al*.,2013** | - *Aspergillus flavus, Aspergillus niger, Fusarium spp* | - contact assay in vitro |
| **Article of Haoui *et al*.,2016** | - *Fusarium culmorum, Fusarium graminearum* | - the agar dilution method |
| **Article of Tolba *et al*., 2015** | - *Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum* | - Disc diffusion method<br>- Disc volatilization method |

| | | |
|---|---|---|
| **Article of Houicher *et al*.,2015** | - *Aspergillus ochraceus* NRRL 3174, *Aspergillus flavus* NRRL 3251), *Fusarium graminearum*MUCL 53452, *Fusariummoniliforme* MUCL 53645, *Penicillium citrinum* MUCL 31475, *Penicillium expansum* MUCL 29192) *Aspergillus parasiticus*CBS 100926). *Penicillium viridicatum, Aspergillus niger, Fusarium culmorum)* | - A broth macrodilution method |

Concerning the used methods to evaluate the antifungal activity, there has been used so many methods that were the same as in some articles while others, used different ones.

All the article of Mehani *et al*., 2015, Mazari *et al*., 2010, Abi-Ayad *et al*., 2013,Yakhlef *et al*., 2020 and Haoui *et al*., 2016, depended on testing the antifungal activity of the EOs by using the direct contact technique though, there was a second applied method by the article of Yakhlef *et al*., 2020 next to the first method one which was the microatmosphere method.

Both of the article of Ksouri *et al*., 2017 and Houicher *et al*., 2015 used the broth macrodilution method.

Another technique which called insemination was used by the article of Belmekki *et al*., 2013.

Three other articles have used the disk diffusion method which were, the article of Dob *et al*., 2006 using a filter paper disk, the article of Kacem *et al*., 2016 and Tolba *et al*., 2015, the later one had another method next to the mentioned one which is the disk volatilization method.

# Chapter 04

# Results and discussion

# Chapter 04 results and discussion

## 4.1. The antifungal activity

### 4.1.1. Article of Mehani *et al*., 2015

Antifungal activity was revealed by the absence or presence of mycelial growth. The results of the EO antifungal activity diameter of *Mentha pepirita* plant have been shown in the figure 1 that shows mycelia growth (mm) of *Fusarium sporotrichioides*, *Fusarium graminearum* and *Fusarium langsethiae* according to the incubation time and the EO concentration of *Mentha pepirita*.

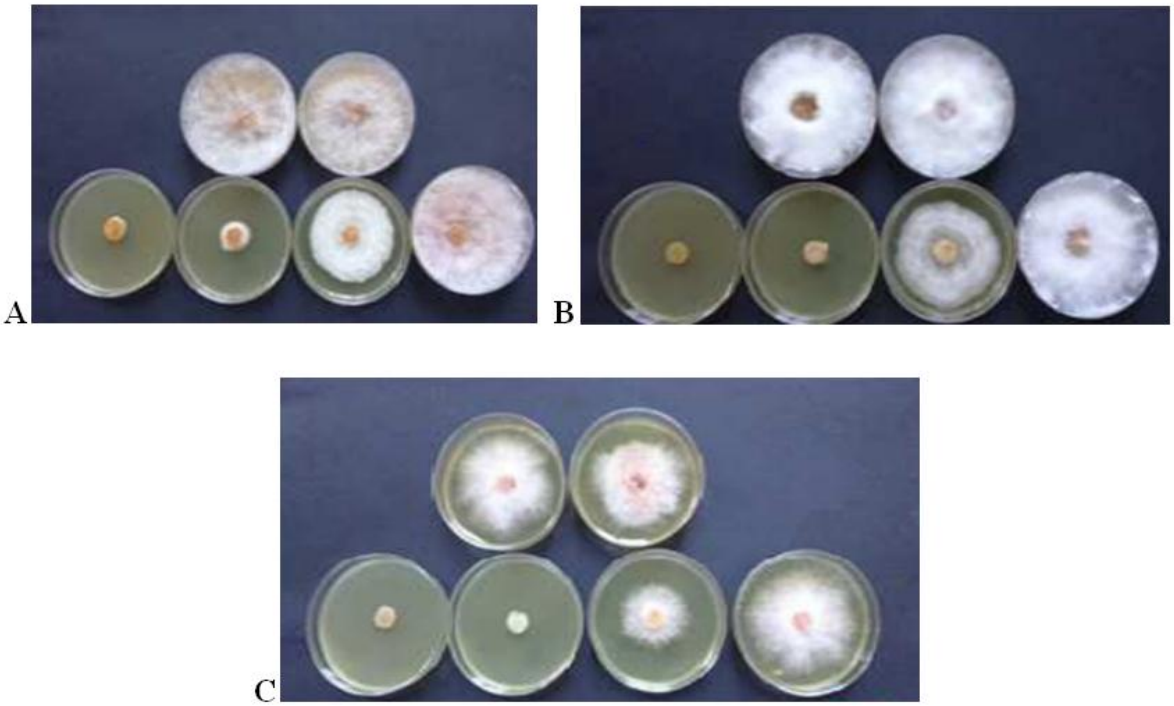


**Figure 1:** Antifungal Activity of Mentha pepirita on A: *Fusarium sporotrichioides*, B: *Fusarium graminearum* and C: *Fusarium langsethiae* (Mehani *et al*., 2015).

With different concentrations of essential oil extracted from *Mentha pepirita* figure 1.A showing that mycelia growth of *Fusarium sporotrichioides* was remarkable after 72 hours for the control and different concentrations of *Mentha pepirita* essential oil: 0.0125, 0.025 and 0,05µl. while figure 1.B which represents the antifungal activity of *Fusarium graminearum* according to the incubation time and the concentration of essential oil of *Mentha pepirita* shows that there is an increase of mycelial growth with the incubation time. In accordance with the figure 1.C that represents the Antifungal activity of *Fusarium langsethiae* With different concentrations of essential oil, where the mycelial growth of the fungi was

remarkable after 72 hours for the control and the different essential oil concentrations the plant 0,0062 and 0.0125 µl by cons 0.025 and 0.05 µl where no mycelial growth of *Fusarium langsethiae* in *Mentha pepirita* were observed.

### 4.1.2. Article of Tabet Zatla *et al.*, 2017

The inhibitory effects of essential oils extracted from roots and aerial parts were evaluated against the three plant fungi named *P. expansum*, *A. niger* and *B.cinerea*, where The data indicated that essential oil of roots at concentration of 70 mL/L produced the greatest inhibition (100%) for *P. expansum* and *B. cinerea*. On the other hand, the essential oils extracted from the aerial parts had no effect against *P. expansum*, *A. niger* and *B. cinerea*.

**Discussion**

This study was conducted to assess the possibility of developing agents to control fungus diseases. The results of the present study suggest that essential oils extracted from roots of *D. carota* subsp. *sativus* have very interesting in vitro antifungal effects against the three pathogens. Herb, flowering umbel and mature umbel oils from *D. carota* subsp. *carota* (collected in their natural habitat near Lodz Poland). They exhibited moderate antifungal activities against *Candida albicans* .This study indicated that root essential oil possess an in vitro antifungal activity against gray mould disease *agent B. cinerea*. The in vivo fungicidal activity of essential oils of *D. Carota* subsp. *sativus* was also investigated in post-harvest conditions. The strawberry fruits (preventive activity: no fungal inoculation and protective activity: after fungal inoculation) were exposed to essential oil, in order to reveal whether essential oil has preventive or protective activities. Although essential oil was effective in reducing of disease severity in treatment preventive and protective during storage period at 24 ± 1°C, greatest activity was observed with essential oil that showed a 100% protection against gray mould with preventive treatment (Figure 2(a)). The antifungal properties of the essential oil may be related to the presence of high concentrations of major compounds or to a synergistic effect with some active components. In general, the active antifungal compounds of essential oils are phenolic compounds.

(a) (b)

**Figure 2:** Preventive effect of root essential oil (a) after seven days of storage at 24 ± 1°c, controls (b). (Tabet Zatla *et al*., 2017).

### 4.1.3. Article of Ksouri *et al*., 2017

The different values of minimum inhibitory concentration (MIC) 80% obtained shows that For *O. floribundum* Munby essential oil, the recorded MIC 80% values against mastitis cases isolated strains and the standard strain range from 17.18 to 23.14% mg/mL. *T. ciliates* Desf. essential oil showed an activity almost comparable to that of *O. floribundum* Munby., with MIC 80% values ranging from 15.02 to 20.96 mg/ml. The antifungal activity of *R. officinalis* essential oil is less than the two previous essential oils, with MIC 80% ranging from 23.99 to 31.08 mg/mL.

**Discussion**

The results of the antifungal activity of *O. floribundum* Munby essential oil demonstrated an appreciable anticandidal activity, with 80% MIC between 17.18 and 23.14 mg/mL. In addition, the tested ATCC strain by the same essential oil showed a MIC 80% value comparable to clinical isolates (18.99 mg/mL). Comparing these results with those of the anticandidal activity tests of the same chemotype oregano species, it is shown that a sample of *Origanum majorana*, collected in June 2004 from Taoudjnia region (Guelma), has a lower value of MIC 80% (1.59 mg/mL). In contrast, Bendahou *et al*., found a higher value of MIC 80% (36 —57 mg/mL), when testing the anticandidal activity of *Origanum glandulosum* Desf. which was harvested in June 2005 from Sebdou region (Western Algeria).

The evaluation of the antifungal activity of *R. officinalis* L. essential oil against ten strains of *C. albicans* clinical isolates, showed MIC 80% values ranging from 23.99 to 31.08 mg/mL. ATCC strain was also tested and showed a MIC 80% value belonging to the same interval (30.27 mg/ mL). These MIC 80% values are very high, when compared with those of *Rosmarinus officinalis* L. (MIC 80% of 2.208 mg/ mL) collected from Annaba in February2003 by(Giordani *et al*., 2003) This variability of antifungal power could be related to seasonal and geographic factors. Antifungal activity of *Thymus ciliates* Desf. essential oil has been highlighted during the tests carried out on ten clinical isolates. The MIC 80% values range from 15.02 to 20.96 mg/mL. These values are close to the MIC 80% value of *C. albicans* standard strain (18.88 mg/mL).

It is important to notice that essential oils are complex mixtures of several compounds; it is difficult to attribute their biological activities to a particular component. Usually, the main compounds are responsible for biological effects.

These results allowed attributing the anticandidal activity of these two essential oils, to the action of their major components. The mechanisms of action of these natural compounds appear to due to the inhibition of the ergosterol biosynthesis and membrane integrity disruption. These compounds could be directly implicated in the significant antifungal activity of Rosemary essential oil. Studies showed that natural compounds act on cellular integrity, respiration inhibition, ion transport processes and increase the permeability of *C. albicans* membranes.

### 4.1.4. Article of Alam *et al*., 2013

In this research the antifungal activity of the three plant species, belonging to 3 botanical families (Lamiaceae, Apiaceae and Cupressaceae) were tested against *F. oxysporum*, *A. solani*, *A. niger*, *Penicilliumsp*1 and *Penicillium sp*2. The effect of plant essential oils depends according to the plant species. In fact, two plant species out of three reduced mycelial growth of *F. oxysporum*, *A. solani*, *A. niger*, *Penicilliumsp*1and *Penicillium sp*2by more than $_{50}$ %. Among these plants *T. capitatus*, belonging to the families of Lamiaceae, completely inhibited mycelial growth of tested fungus. *T. capitatus* essential oil produced the greatest reduction in mycelium growth with these fungi at 2 µg mL-1, with percentage reductions of 100 %. The second most effective essential oil with this five fungi was *T. articulata* essential oil, with percentage of mycelial reduction in *F. oxysporum*, *A. solani*, *A. niger*, *penicillium sp*1 and *penicillium sp*2 of 36.11, 35.12, 11.11, 34.56 and 45.12 %,respectively, at the same concentration. However, the data indicates that the percentage

inhibition of mycelial growth increased with increasing concentration of essential oils for all strains tested, suggesting that the essential oil of *T. articulata* inhibited the growth of all strains in a dose-dependent manner. Essential oil *D. crinitus* cause no percentage of mycelial reduction, except against *penicillium sp*2. This activity was more pronounced, where the percentage of inhibition increased to 54.32 % at 2 µg mL-1, reaching a maximum of 77.77 % at 5 µg mL-1, suggesting that this strain was the most sensitive to the essential oil.

**Discussion**

The mycelial growth of colonies in the presence of the essential oil of *T. capitatus* and *T. articulate* showed that it effectively controlled all the fungi tested. The mycelia growth of colonies in the presence of the essential oil of *T. capitatus*and *T. articulata* showed that it effectively controlled all the fungi tested. This efficiency can be explained by the presence of active molecules that inhibited the growth of the five phytopathogenic fungi. This activity may be produced by a single major compound or by the synergistic or antagonistic effect of various compounds (DEBA *et al*., 2008).

### 4.1.5. Article of Elhouiti *et al*., 2022

Kinetics of fungal growth of *Fusarium* strains in the presence of different concentrations of the tested plant EOs showed a remarkable sensitivity of the strains of *F. oxysporum*. The two strains BD17 and INRA 349 were found to be resistant to low concentrations of EOs from *T. algeriensis, A.campestris* and *A. herba-alba*, sensitive to EOs of *M. pulegium* and *M. piperita*, very sensitive to the EO of *T. vulgaris*. At the minimum concentration tested 0.25 µl/ml of *T. vulgaris* essential oil, the growth of FOA, FOL and FOP strains of *F. oxysporum* was completely inhibited. The $IC_{50}$ (inhibitory concentration of $_{50}$ % of mycelia growth calculated graphically by linear regression) and MIC values show very high bioactivity of *T. vulgaris* EO against all strains. In general, $IC_{50}$ values of the other EOs are between 0.01 and 0.6 µl/ml against the strains of *F. oxysporum* which are more sensitive to *T. vulgaris* and *A. campestris* essential oils, on the other hand, the strains of *F. culmorum* and *F. graminearum* are only sensitive in the presence of *T. vulgaris* EO. A weak inhibitory effect of EOs of *A. campestris* and *A. herba-alba* compared to other oils resulted in high MIC values against the strains BD17, INRA 349, FOA and FOP. Potency and efficiency of EOs of *M. pulegium* and *M. piperita* were important especially against the strains of *F. oxysporum*. Against strains BD17 and INRA 349, $IC_{50}$ and MIC values recorded by these tested Eos are more effective than the EO of *Rhanterium adpressum* (0.7 and 1.54 for $IC_{50}$, 12.81 and 12.73µl/ml for MIC for the two strains respectively).

### 4.1.6. Article of Belmekki *et al*., 2013

The results of antifungal activity assays showed that the oils moderately reduced the growth of *A. flavus* (25.9% inhibition at 10 µl/ml of essential oil). As well only 10.53% of inhibition was observed against *F. oxysporum* at 10 µl/ml of essential oil. On the other hand, the oil was significantly not active against *R. stolonifer* and that even with 10 µl/ml of essential oil. Inhibitory effects of the oil on the growth of fungal strains were lower compared to amphotericin B.

### 4.1.7. Article of Dob *et al*., 2006

This screening of oil antifungal activity using the paper disk diffusion method showed that the oil has inhibited the growth of all filamentous fungi tested (*F. oxysporum sp. Albedinis* and *M. ramanianus*), as also the growth of all yeast (*S. cerevisiae* and *C. albicans*). The oil presents the best action as antifungal, in which *F. oxysporum sp. Albedinis* was the most susceptible fungi (inhibition zone $_{50}$ mm).

The used antifungal method of *T. fontanesii* oil has been approved to be such a strong antifungal especially against the date palm blight pathogen (fusariose) *F. oxysporum sp. albedinis* (Kabouche *et al*., 2005). The antifungal activity maybe therefore due to the biological components that the EOs are composite of such as terpenoids, esters, phenols, ketones, acids, aldehydes..etc., some of the biological components were regarded as membrane destructors (interference with the membrane enzymes) by (Knobloch *et al*., 1989).

### 4.1.8. Article of Mazari *et al*., 2010

After the isolation of *J. phoenicea* and *C. sempervirens* EOs from leaves in the goal of testing their antifungal activity on the three fungal strains and their fungistatic effects were compared with the commercial antifungal amphotericin B.

The results of antifungal activity assays showed that the oils moderately reduced the growth of *Aspergillus flavus* and *Fusarium oxysporum*. However, the oils were significantly not active against *Rhizopus stolonifer*. Inhibitory effects of the oils on the growth of fungal strains were lower compared to amphotericin B. As seen from Table, the oils exhibited similar inhibition effects on the growth of tested fungi, which might be attributed to their similar compositions.

The antimicrobial activity of the essential oils of *J. phoenicea* and *C. sempervirens* could, in part, be associated with theirs major constituents such as α- pinene, β-phellandrene,

α- Terpinyl acetate and cedrol. These components have been reported to display antimicrobial effects (Yang *et al*., 2007; Demirci *et al*., 2007).

### 4.1.9. Article of Yakhlef *et al*., 2020

Depending to the obtained results it is clear that the EO has a significant antifungal activity, it was more marked through the volatile fraction of the EO tested by the micro atmosphere method (MA) compared to its liquid fraction tested by direct contact in an agar medium (SM) which gave respectively $IC_{50}$ of 0.092 ± 0.006 and 0.254 ± 0.031.

The explanation of these observations can be attributed, to the difference in composition between the liquid phase of the EO, in the microdilution method in agar medium and the vapor phase implemented in the micro-atmosphere technique. On the other hand, the low solubility of essential oils in polar media makes it harder for them to diffuse onto the agar medium, because of their hydrophobic character. Indeed, the volatile and hydrophobic character makes these oils more absorbable by the fungal agent than by direct contact on agar (Laghchimi *et al*., 2014).The antifungal effect of *M. rotundifolia* EO is probably due to its chemical composition rich in oxygenated monoterpenes (52.47 %). These compounds are known for their ability to inhibit mycelial growth.

On the other hand, the antifungal activity of EO may be due to the presence of caryophyllene oxide (oxygenated sesquiterpene with 35.27 % of the overall composition of the oil). This major compound is known for its use as a preservative in foods, drugs and cosmetics (Yang *et al*., 2007).

### 4.1.10. Article of Kacem *et al*., 2016

*Genista quadriflora* EO exhibited significant activity against fungi *F. oxysporun*. The lipophilic character of the terpene skeleton combined to the hydrophilic character of the functional groups is essential for activity that ranked as follows: aldehydes > ketones > alcohols > esters > hydrocarbons. With a high amount of terpenoids (66.85%), *G. quadriflora* EO can be suggested as potent active oil against invasive fungal infections inimmunosuppressed patients due to *F. oxysparum*. Furthermore, EO contains compounds of interesting biological properties.

### 4.1.11. Article of Benomari *et al*., 2017

The Algerian *Mentha* species EO effect was tested on for fungi. *Monilinia sp*. and *B. cinerea* strains MICs showed more sensitivity to the EO compared to *P. expensum* that was less sensitive.

Essential oils of *M. rotundifulia* are less active than the essential oils from other species of *Mentha*, except for MRO3 which has an MIC of 36 × 103 µl/ml air against *B. cinerea*. They observed an MIC between 36 and 142 × $10^3$ µl/ml air for *M. spicata, M. pulegium*, and *M. piperita*. These results can be correlated with both their chemical compositions and statistical results. Oils dominated by alcohols (MPI1 and MPI2 and MPU3), aldehydes and ketones (MRO3, MSP1, MSP2, MPU1, MPU2, and MPU3) exhibited an interesting inhibition again the fungi tested, in contrast to MRO1 and MRO2 which were rich in oxide compounds and were less active.

### 4.1.12. Article of Abi-Ayad *et al*., 2013

The results of antifungal activity assays showed that the EO extracted from the *T. articulata* had an inhibitory effect on the growth of fungi.

Percentage of growth inhibition was influenced by incubation time and essential oil concentration. The more concentration of the EO is high the more the inhibition rate is high either, while it is not the same thing considering the incubation time; the results showed that the mycelia growth was considerably increased with incubation time for *Fusarium spp*. and decreased with incubation time for *A. niger, Aspergillus flavus* showed the same effect as *A. niger*.

*Fusarium spp*. was most suppressed as its growth was completely inhibited by 20 µl/ml the 6 day of incubation and was reduced to 91 % after 8 days of incubation, followed by *A. flavus* and *A. niger*. At the concentration of 15 µl/ml, the essential oil appeared to be active against growth of the three pathogens above 60 %.

The antifungal inhibitory effect in their study on the three fungi may be due to the chemical compounds of the EO itself

The present study has thus shown that this essential oil may have potential use against *A. flavus, A. niger* and *Fusarium spp*. In general, the inhibitory action of natural products on fungal cells involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of synthesis of intracellular and extracellular enzymes.

### 4.1.13. Article of Haoui *et al*., 2016

In this study, three fractions of essential oils were chosen to evaluate their antifungal effect: fraction 1 (90-120 min); fraction 2 (210-240 min) and the total essential oil (0-300 min). The results noticed that for the various concentrations, the essential oil of each fraction was very effective (between 62.5 - 91.25 %). The inhibitions of the mentioned fractions are probably due to the chemical constituents they may contain. Thus the results show that it will be enough to use the first fraction 1 of *I. viscose* EO as a natural fungicide.

### 4.1.14. Article of Tolba *et al*., 2015

**Disc diffusion method**

Based on the obtained results, *E. citriodora* essential oil successfully inhibited the growth of all dermatophytes tested in the present study. The zone of inhibition increased with the increasing concentration (i.e 10, 20, 30 mL) of the oil. The highest inhibition zone value was observed against *Microsporum canis* (64 mm), *Trichophyton mentagrophytes* (64 mm), *Trichophyton rubrum* (39 mm) by using 10 mL of the oil, such that it inhibits growth absolutely of the three dermatophytes at 20 and 30 mL of the oil, while *Microsporum gypseum* was the most resistant to the oil with inhibition zone from (10, 20 mL) of the oil was 12 mm and 29.5 mm at 30 mL of the oil.

**Disc volatilization method**

The antifungal activity of *E. citriodora* essential oil was also evaluated in the vapour phase.

Asobserved in the liquid phase, the zone of inhibition due to the oil vapor also increased with increasing concentration of the oil. Further, *Microsporum canis*, *Trichophyton mentagrophytes* were completely inhibited by the oil vapours at 20 and 30 mL, while *Trichophyton rubrum* and *Microsporumgypseum* formed a 39-mm and 24-mm inhibition zone, respectively, from the vapours of 30 mL *E. citriodora* essential oil. *Microsporum gypseum* was more resistant to the oil vapour at 10 and 20 mL. For all the tested strains, the inhibition zone resulting from exposure to *E. citriodora* essential oil vapours was less than that resulting from the same concentration of the oil in liquid phase.

### 4.1.15. Article of Houicher *et al*.,2015

*Fusarium graminearum* was the most sensitive strain to *A. campestris* essential oil with MIC and MFC values of 1.25 µl/ml (v/v). This oil showed a potent inhibitory effect against *F. moniliforme*, *F. culmorum*, *P. expansum*, *A. flavus*, *A. ochraceus*, and *A. parasitcus* with MIC

value of 2.5 µl/ml (v/v). *Penicillium viridicatum* and *Aspergillus niger* was the most resistant fungi to *A. campestris* essential oil (MIC=10 µl/ml) while the growth of *P. citrinum* was effectively inhibited at MIC value of 5 µl/ml (v/v). It must be noted that MIC values of *A. campestris* essential oil showed a variability among all the tested fungi (1.25–10 µl/ml) which could be related mainly to the fungal specie. The essential oil also exhibited strong fungicidal activity against the tested fungi, except for *P. citrinum*, *P.viridicatum* and *A.niger* (MFC >2 µl/ml). The oil concentration of 2.5µl/ml (v/v) exhibited fungicidal activity against *F. moniliforme, F. culmorum, P. expansum,* and *A. flavus* whereas 5 µL/mL (v/v) was enough to exert a fungicidal effect against *A. parasitcus* and *A. ochraceus*.

# Conclusion

## Conclusion

In this review, 15 research articles about the antifungal activity of essential oils were analyzed. Several plants belonging to the Algerian flora were studied. More than 20 fungi strains (pathogenic and not pathogenic) were tested.

The plant species belongs mainly to the Lamiaceae, Apiaceae and Asteraceae families.The major parts of fungi strains are moulds, in some cases, yeasts were tested, especially *Candida albicans*.

The antifungal assays were carried out by the following methods : agar incorporation method, broth macrodilution in tubes, direct contact diffusion method using filter paper disk, in addition to another disc volatilization method.

Based on the obtained results in the different articles, antifungal activity assays showed that several EO extracted from the Algerian plants had an inhibitory effect on the growth of fungi.

Percentage of growth inhibition was influenced by incubation time and essential oil concentration. When the concentration of the EO increases, the inhibition rate is high either.

Inhibition zone resulting from exposure to essential oil vapours was less than that resulting from the same concentration of the oil in liquid phase.

In general, the inhibitory action of natural products on fungal cells involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of synthesis of intracellular and extracellular enzymes.

It is important to notice that essential oils are complex mixtures of several compounds; it is difficult to attribute their biological activities to a particular component. Usually, the main compounds are responsible for biological effects. The effect of plant essential oils depends on the plant species. The antifungal activity maybe therefore due to the biological components that the EOs are composite of such as terpenoids, esters, phenols, ketones, acids, aldehydes…..etc.

# References list

## References list


1. **Abi-Ayad F. Z., Abi-Ayad M., Lazouni H., A., Rebiahi S. A. (2013).** Evaluation of *Tetraclinis articulata* essential oil from Algeria flora as a potential source of antifungal activity and study of its chemical composition .Journal of the Indian Academy of Wood Science, 10(1), 9-15.

2. **Alam S. B., Benyelles N. G., Dib M. E. A., Djabou N., Tabti L., Paolini J., Costa J. (2014).** Antifungal activity of essential oils of three aromatic plants from western Algeria against five fungal pathogens of tomato (*Lycopersicon esculentum* Milll). Journal of Applied Botany and Food Quality, 87.

3. **Bar-Hen A., Mariadassou M., Poursat M. A., Vandenkoornhuyse P. (2008).** Influence function for robust phylogenetic reconstructions. Molecular Biology and Evolution. 25:869–873.

4. **Beirão A. R. B. et Gil M. G. (2006).** Antioxidants from *Lavandula luisieri*. 2 nd Mercosur Congress on Chemical Engineering. Portugal. 8p.

5. **Bekechechi C., Abdelouahid D. (2014).** Les huiles essentielles. Ed Office des publications Universitaire, Alger. 43p.

6. **Belaiche P. (1979).** Traité de phytothérapie et l'aromatothérapie. Tome I: L'aromatogramme. Ed.maloine S., Paris, p. 204.

7. **Benomari, F. Z., Andreu V., Kotarba J., Dib M. E. A., Bertrand C., Muselli A., Djabou N. (2018).** Essential oils from Algerian species of *Mentha* as new bio-control agents against phytopathogen strains .Environmental Science and Pollution Research, 25(30), 29889-29900.

8. **Boiron P. (1996).** Organisation et biologie des champignons. Edition Nathan. P: 13-19-69-79.

9. **Botton B., Beton A., Fevre M., Gauthier S ., Guy P ., Larpent J. P., Reymond P., Sanglier J. J., Vayssiery Y., Veau P. (1990).** Moisissures utiles et nuisibles importance industrielle. 2 ème édition. Masson .Collection Biotechnologies .P : 34-428.

10. **Bouchet P. H., Guignard J. L. et Villard J. (1999).** Les champignons. Mycologie fondamentale et appliquée. Edition Masson. 194p.

**11. Bruneton J. (1999).** Pharmacognosie. Phytochimie, plantes médicinales. 3 ème édition, Tec & Doc. Lavoisier, Paris, 1120p.

**12. Bruneton J. (1999).** Plantes toxiques végétaux dangereux pour l'homme et les animaux. Ed Tec et Doc, Paris. 83p.

**13. Capo M., Courilleau V. et Valette. (1990).** Chimie des couleurs et des odeurs. Culture et techniques, p. 204.

**14. Carson C. F., Rilley T.V. et Bosque F. (2002).** Antimicrobial activity of the major components of essential oil of *Malaleuca alternifolia*. Journal of Applied Bacteriology. 78, p:264-269.

**15. Castegnaro M., Pfohl-Leszkowicz A. (2002).** Les mycotoxines : contaminants omniprésents dans l'alimentation animale et humaine, dans La sécurité alimentaire du consommateur, Lavoisier, Tec&Doc. Conserv, 7, 1147-1161.

**16. Charpentier B., Hamon-lorleac'h F., Harlay A., Huard A., Ridoux L., Chanselle S. (2008).** Guide du préparateur en pharmacie. 3éme édition, Elsevier Masson, 1358.

**17. Cox S. C., Mann J., Markham H., Bell J., Gustafson J., Warmington, and Wyllie S. (2000).** The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). Journal of applied microbiology, 88(1): p. 170-175.

**18. Davidson P.M. (1997).** Methods for testing the efficacity of food antimicrobial. Food Technology.43, p:148-155.

**19. Defeo V., Bruno M., Tahiri B., Napolitano F. and Senatore F. (2003).** Chemical composition and antibacterial activity of essential oils from *Thymus spinulosis* Ten (Lamiaceae). J. Agric. Chem., N° 51, pp. 3849-3853

**20. Dob T., Dahmane D., Benabdelkader T., Chelghoum C. (2006).** Composition and Antimicrobial Activity of the Essential Oil of *Thymus fontanesii* .Pharmaceutical biology, 44(8), 607-612.

**21. Dorman H. J. D. et. Deans S. G. (2000).** Antimicrobial agents from plants: antibacterial activity of plant volatile oil, Journal of Applied Microbiology, 88: 308 - 316.

**22. Duraffourd C., D'Hervicourt L. et Lapraz J. C. (1990).** Cahiers de phytothérapie clinique. 1. Examens de laboratoires galénique. Eléments thérapeutiques synergiques. 2èmeéd. Masson, Paris.

**23. Elhouiti F., Benabed K. H., Tahri D., Ouinten M., Yousfi M. (2022).** Antioxidant and antifungal activities of essential oils from Algerian spontaneous plants against five strains of *Fusarium spp*. Protection, 30.

**24. Encarta. (2005).** Microsoft corporation 1993-2004.

**25. Ferhat M. A., Meklatib. Y., Chematf. (2007).** Comparison of different isolation methods of essential oil from Citrus fruits cold pressing, hydrodistillation and microwave « Dry » distillation. FLAVOUR fRragr. J .22/ p 494-$_{50}$4.

**26. Fournier ET Bonderf. (1983).** Les produits antiparasitaires à usage agricole (conditions d'utilisation). 316p.

**27. Frazier W. C. (1967).** Food microbiology. Academic presse. London. P : 3-429.

**28. Haoui I. E., Derriche R., Madani L., Oukali Z. (2016).** Extraction of essential oil from *Inula viscosa* (L.) leaves: composition, antifungal activity and kinetic data .Journal of Essential Oil Bearing Plants,19(1),108-118.

**29. Houicher A., Hechachna H., Özogul F. (2016).** In vitro determination of the antifungal activity of *Artemisia campestris* essential oil from Algeria .International Journal of Food Properties, 19(8), 1749-1756.

**30. Hussain A. I. (2009).** Characterization and biological activities of essential oils of some species of lamiaceae. Thése de Doctorat. Pakistan. p257.

**31. Hussain AI., Anwar F., Chatha SAS., Jabbar A., Mahboob S. et Nigam P. S. (2010).** *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. Brazilian Journal of Microbiology. 41 p: 1070-1078.

**32. James T. Y., Porter D., Leander C. A., Vilgalys R., Longcore J. E. (2000).** Molecular phylogenetics of the Chytridiomycota supports the utility of ultrastructural data in chytrid systematics. *Canadian* Journal ofBotany. 78: 336-3$_{50}$.

**33. Jennings D.H., Lysek G. (1996).** Fungal Biology: understanding the fungal lifestyle. Bios Scientific publishers eds.

**34. Kabak B., Dobson A. D., Var I. I. L. (2006).** Strategies to prevent mycotoxin contamination of food and animal feed: a review .Critical reviews in food science and nutritio, 46(8), 593-619.

**35. Kacem N., Roumy V., Duhal N., Merouane F., Neut C., Christen P., Rhouati S. (2016).** Chemical composition of the essential oil from Algerian *Genista quadriflora* Munby and determination of its antibacterial and antifungal activities .Industrial crops and products, 90, 87-93.

**36. Khadidja M., Nassima B., Chahrazed B., Xavier F. (2010).** Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L. Journal of Medicinal Plants Research, 4(10), 959-964.

**37. Ksouri S., Djebir S., Bentorki A. A., Gouri A., Hadef Y., Benakhla A. (2017).** Antifungal activity of essential oils extract from *Origanum floribundum* Munby, *Rosmarinus officinalis* L. and *Thymus ciliatus* Desf. against *Candida albicans* isolated from bovine clinical mastitis .Journal de mycologie médicale, 27(2), 245-249.

**38. Lis-Balchin M., Steyrl H., Krenn E. (2003).** The comparative effect of novel Pelargonium essential oils and their corresponding hydrosols as antimicrobial agents in a model food system . Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 17(1), 60-65.

**39. LUCCHESI M.E. (2005).** Extraction Sans Solvaont Assistée par micro-ondes Conception et Application à l'extraction des huiles essentielles, thèse doctorat : Université de la Reunion, Faculté des Sciences et Technologies France, p146.

**40. Maslin J., Morand J. J., Menard G., Camparo, P. (2004).** Aspergillosis .Medecine tropicale: revue du Corps de sante colonial, 64(1), 11-17.

**41. Mehani M., Segni, L., Terzi, V., Morcia, C., Ghizzoni R., Goudjil M. B., Bencheikh S. E. (2015).** Antibacterial, antifungal activity and chemical composition study of essential oil of *Mentha pepirita* from the south Algerian. Der Pharma Chemica, 7(12), 382-387.

**42. Mishra K. K., Kaur, C. D. , Sahu A. K., Panik R., Kashyap P., Mishra, S. P., Dutta, S. (2020).** Medicinal Plants Having Antifungal Properties. In (Ed.), Medicinal Plants - Use in Prevention and Treatment of Diseases.

**43. Morgavi D. P., Riley R. T. (2007).** *Fusarium* and their toxins: mycology, occurrence, toxicity, control and economic impact. Animal Feed Science and Technology, 137(3), 199-200.

**44. Moro - Buronzo A. (2008).** Grand guide des huiles essentielles : Santé, Beauté, Bien- Etre, Hachette pratique. 14.

**45. Nacéra B., Nassima B., Chahrazed B., Xavier F. (2013).** Chemical analysis and antimicrobial activity of *Teucrium polium* L. essential oil from Western Algeria .Journal of Medicinal Plants Research, 7(14), 897-902.

**46. Piškur J., Rozpędowska E., Polakova S., Merico A., Compagno C. (2006).** How did Saccharomyces evolve to become a good brewer?. TRENDS in Genetics, 22(4), 183-186.

**47. Porter N. (2001).** Essential oils and their production.Crop & Food Research, p.39.

**48. Powell M. J. (1993).** Looking at mycology with a Janus face: a glimpse at Chytridiomycetes active in the environment. Mycologia. 85: 1-20.

**49. Punt P. J., Van Biezen N., Conesa A., Albers A., Mangnus J., Van den Hondel C. (2002).** Filamentous fungi as cell factories for heterologous protein production. Trends Biotechnol. 20 (5): 200-206.

**50. Rai M., Acharya D. et Wadegaonkar P. (2003).** Plant derived-antimycotics: potential of Acteraceous plants. In: Plantb-derived antimycotics: Current trends and future prospects, HaworthPress, N-York, Londin, Oxford, p.165-185 .

**51. Reboux G. (2006).** Mycotoxins: health effects and relationship to other organic compounds. Revue Française d'Allergologie et d'Immunologie Clinique 46, 208-212.

**52. Santos M. A., Mateos L., Stahmann K. P. Revuelta J. L. (2005).** Insertional mutagenesis in the vitamin B 2 producer fungus *Ashbya gossypii*. In Microbial Processes and Products (pp.283-300).Humana Press.

**53. Semal., Fraselle J., Impens R., Kummert J., Le poivre P., Meulimans M., Seilleur P., Vendrevenen J et Viseur J. (1993).** Traite de pathologie végétale .Presse agronomique de Gembloux Belgique. pp178, 181,185, 186,194**.**

**54. Tabet Zatla A., Dib M. E. A., Djabou N., Ilias F., Costa J., Muselli A. (2017).** Antifungal activities of essential oils and hydrosol extracts of *Daucus carota* subsp. *sativus* for the control of fungal pathogens, in particular gray rot of strawberry during storage. Journal of EssEntial oil rEsEarch, 29(5), 391-399.

**55. Tabuc C. (2007).** Flore fongique de différents substrats et condition optimales de production des mycotoxines. Pathologie et Génétique. Université de Bucarest P: 16-57.

**56. Taylor J. W., Jacobson D. J., Kroken S., Kasuga T., Geiser D. M., Hibbet D. S., Fisher M. C. (2000).** Phylogenetic species recognition and species concept in fungi. Fungal Genetics and Biology. 31: 21-32.

**57. Teusher E., Anton R., Lobstein A. (2005).** Plantes aromatiques. Eplices, aromates, condiments et huiles essentielles. Tec & Doc, Paris, 522**.**

**58. Tisserand R. and Young R. (2014).** Essential Oil Safety: A Guide for Health Care Professionals. Elsevier Health Sciences, United Kingdom. 783 p.

**59. Tolba H., Moghrani H., Benelmouffok A., Kellou D., Maachi R. (2015).** Essential oil of Algerian *Eucalyptus citriodora*: Chemical composition, antifungal activity .Journal de mycologie medicale ,(4)25 ,e128-e133.

**60. Willem J. P. (2002).** Le guide des huiles essentielles pour vaincre vos problèmes de santé. Editions LMV.

**61. Willem J. P. (2013).** 60 maux soignés par les huiles essentielles : l'aromathérapie au quotidien pour toute la famille, Les mini pockets de santé.

**62. Wu, F. (2006).** Mycotoxin reduction in Bt corn: potential economic, health, and regulatory impacts .Transgenic research, 15(3), 277-289.

**63. Yakhlef G., Hambaba L., Pinto D. C., Silva A. M. (2020).** Chemical composition and insecticidal, repellent and antifungal activities of essential oil of *Mentha rotundifolia* (L.) from Algeria .Industrial crops and products, 158, 112988.

**ملخص**

تحدث الالتهابات الفطرية بسبب كائنات حقيقية النواة ، وبالتالي يصعب التأكد من وجودها وتطبيق العلاج العلاجي المناسب مقارنة بالعدوى البكتيرية. تتمتع القارة الأفريقية بتنوع بيولوجي غني بين النباتات في العالم، مع وجود عدد كبير جدًامن النباتات المستخدمة كأعشاب ، ويتكون هذاالعمل من مراجعة 15 مقالة بحثية حول النشاط المضاد للفطريات للزيوت الأساسية ، وجميع المقالات المختارة تمت دراستها نباتات تنتمي للنباتات الجزائرية تم تطوير ومقارنة المواد والأساليب والنتائج والمناقشات. سمحت نتائج هذه المقالات أن تنسب النشاط المضاد للفطريات للزيوت الأساسية للنباتات المختلفة لعمل مكوناتها الرئيسية. تظهر آليات عمل هذه المركبات الطبيعية بسبب تثبيط بعض الوظائف الحيوية للفطريات من المهم ملاحظة أن الزيوت الأساسية عبارة عن مخاليط معقدة من عدة مركبات ؛ من الصعب أن تنسب أنشطتهم البيولوجية إلى مكون معين عادة ما تكون المركبات الرئيسية مسؤولة عن التأثيرات البيولوجية.

**كلمات مفتاحية** : فطريات, نباتات جزائرية, زيوت اساسية, نشاط مضاد للفطر.

**Résumé**

Les infections fongiques sont causées par des organismes eucaryotes, et il est donc plus difficile de s'assurer de leur présence et d'appliquer le traitement thérapeutique approprié par rapport aux infections bactériennes. Le continent africain possède une riche biodiversité parmi les plantes du monde, avec un très grand nombre de plantes utilisées comme herbes, Ce travail consiste en une revue de 15 articles de recherche sur l'activité antifongique des huiles essentielles, tous les articles choisis étudiés appartenant à des plantes appartenant à la flore algérienne. Le matériel, les méthodes, les résultats et les discussions sont développés et comparés. Les résultats de ces articles ont permis d'attribuer l'activité antifongique des huiles essentielles des différentes plantes. À l'action de leurs principaux composants. Les mécanismes d'action de ces composés naturels semblent dus à l'inhibition de certaines fonctions vitales des champignons. Il est important de noter que les huiles essentielles sont des mélanges complexes de plusieurs composés ; il est difficile d'attribuer leurs activités biologiques à un composant particulier. Habituellement, les principaux composés sont responsables d'effets biologiques

**Mots clés :** champignons, flore algérienne, huiles essentielles, activité antifongique.

**Abstract**

Fungal infections are caused by eukaryotic organisms, and it is therefore more difficult to ascertain their presence and apply the appropriate therapeutic treatment compared to bacterial infections. The African continent has a rich biodiversity among plants in the world, with a very high number of plants used as herbs. This work consists on a review of 15 research articles about the antifungal activity of essential oils, all of the chosen articles studied plants belonging to the Algerian flora. The material, methods, results and discussions are developed and compared. The results of these articles allowed attributing the antifungal activity of the essential oils of the different plants, to the action of their major components. The mechanisms of action of these natural compounds appear to due to the inhibition of some vital functions of the fungi. It is important to notice that essential oils are complex mixtures of several compounds; it is difficult to attribute their biological activities to a particular component. Usually, the main compounds are responsible for biological effects.

**Keywords:** fungus, Algerian flora, essential oils, antifungal activity.