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Study of the antifungal activity of three plant's extracts against plant pathogenic fungi

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Dedication

I humbly dedicate this work to the souls who have carried me, believed in me, and stood beside me through every step of this journey:

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BOUCHAIR Nesrine

Summary

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General introduction

General introduction

Plants are the main source of essential nutrients for humans, providing vital macro and micronutrients either directly or indirectly through the food chain. Through photosynthesis, they convert soil nutrients into complex organic compounds like proteins, vitamins, and minerals, essential for human health. Key minerals such as iron, zinc, calcium, and potassium obtained mainly from plant based foods support critical physiological functions, and their deficiency can cause serious health issues **(El Ramady & *al*., 2022).**

For millions of years, plants and microbes have developed intricate relationships that influence plant health, soil fertility, and overall ecosystem stability. These interactions vary widely, ranging from mutualistic partnerships, where both organisms benefit, to parasitic associations, where microbes exploit plant resources, often leading to stress or disease. Pathogenic microbes use various strategies to bypass plant defenses and establish infections, ultimately disrupting normal physiological processes **(Williams & *al*., 2024).**

Plant diseases represent a significant threat to global agriculture, causing severe economic damage and undermining food security. On a global scale, these diseases are responsible for the loss of over 30% of crop yields annually, amounting to financial losses worth hundreds of billions of dollars. They reduce both yield and product quality, impacting market value and trade. Additionally, certain pathogens are known to produce toxic substances that contaminate crops, further diminishing their value and posing serious health risks to both humans and animals. To combat these losses, farmers are often forced to increase production inputs such as pesticides and fertilizers, as well as allocate more labor, which significantly raises overall costs. These rising expenses, combined with reduced income from lower-quality produce, place immense pressure on farmers' livelihoods. In the context of a growing global population and increasing demand for food, the economic burden caused by plant diseases continues to escalate, highlighting the urgent need for sustainable and effective disease management strategies in agriculture **(Gai & Wang, 2024).**

Fungal pathogens are increasingly recognized as one of the most significant threats to global agricultural systems, directly contributing to major crop losses and posing a serious challenge to food security worldwide. For example, wheat rust causes $4.3 to $5 billion in global

losses annually. The rise of fungicide-resistant strains and fungi adapting to new environments complicates disease management, highlighting the need for integrated pest management and sustainable control strategies **(Belekar & *al*., 2024).**

Fusarium is recognized as one of the most impactful fungal genera in agriculture, primarily due to its ability to infect a wide range of economically important crops and produce a variety of harmful mycotoxins. These toxins, such as trichothecenes, fumonisins, and zearalenone, are often generated before harvest and contaminate cereal grains worldwide, posing serious threats to food safety. The presence of these mycotoxins in food or feed can cause both acute and chronic illnesses in humans and animals, including, in severe cases, death **(Ekwomadu & Mwanza, 2023).** *Fusarium* (**Figs. 1a, 1b**), can spread through infected seeds, soil, and plant residues, and environmental conditions such as water activity, temperature, light, and timing of infection (particularly around flowering stages) play critical roles in their successful colonization and the accumulation of harmful mycotoxins in plants tissus **(Stępień, 2020).**

Figure 1 : Symptoms of *Fusarium* on tomato **(a) [1],** and on wheat plant **(b)[2].**

Aspergillus niger (black mold), a filamentous ascomycete known for its rapid growth and pH tolerance, is one of the most important cosmopolitan fungi associated with postharvest decay of various substrates. This fungus causes characteristic black mold symptoms on onion bulbs (**Fig. 2**), which are a common host for postharvest infection. This organism is a soil saprobe with

a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocelluloses, and cause decay in a variety of organic substances, including fruits, vegetables, beans, cereals, herbs, wood, … **(Gautam & *al.*, 2011).**

Figure 2 : Black mold caused by *Aspergillus niger* on onions **[3].**

Botrytis, is also one of the economically significant fungal taxa, especially due to its impact on agriculture **(Garfinkel, 2021).** *Botrytis cinerea* (**Fig. 3a, 3b**), also known as grey mould, is a necrotrophic fungus responsible for severe pre- and post-harvest diseases in more than 200 plant species. It affects several economically important crops, including grapevine, tomato, strawberry, cucumber, bulb flowers, cut flowers, and ornamental plants. By inducing host cell death, the pathogen causes extensive damage to plant tissues, leading to significant losses both in the field and during storage **(Van kan, 2006).**

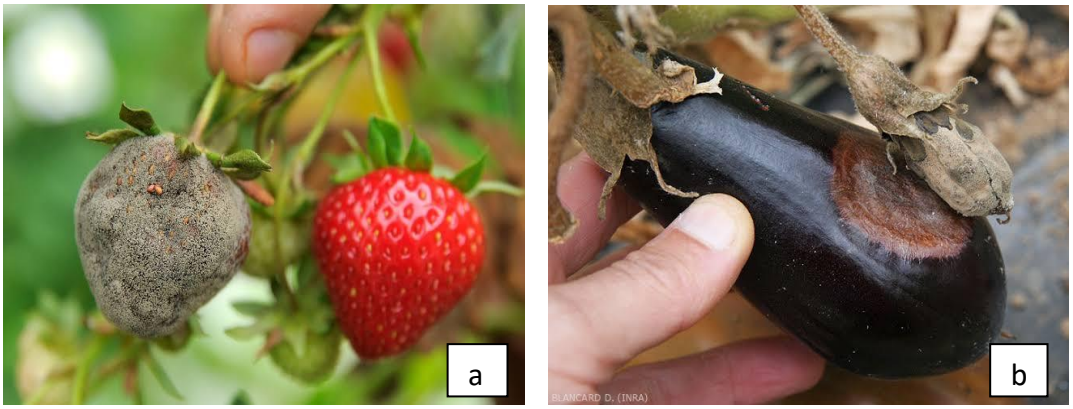

Figure 3 : Symptoms of *Botrytis cinerea* on strawberries **(a)[4],** and eggplant **(b)[5].**

Septoria is a genus of plant pathogenic fungi that is widely distributed across various regions and is frequently linked to leaf spots and stem cankers on a diverse range of plant species. According to **Fones & Gurr (2015),** the pathogen responsible for *Septoria tritici blotch* (STB) is one of the most destructive diseases affecting wheat**.** As illustrated in figure **4**, symptoms of STB on wheat typically manifest as necrotic lesions on leaves, often surrounded by yellow haloes, significantly affecting photosynthesis and plant vitality. This plant pathogenic fungus affects wheat globally and has the potential to cause annual yield losses of up to 50% in wheat-producing regions.

Figure 4 : Symptoms of *Septoria tritici blotch* (STB) on wheat leaves **[6].**

Fungal diseases are a major challenge in crop production, with fungicides being widely used to control them. However, over-reliance on fungicides has led to resistance, reducing their effectiveness and increasing dependence on them. Biological control options remain ineffective, and while resistant cultivars exist, their resistance often breaks down. This highlights the difficulty of moving away from chemical systems and the need for integrated approaches to reduce environmental impact and rethink crop protection strategies **(Finger & *al*., 2024).**

The overuse of synthetic pesticides in agriculture has also led to serious environmental, health, and ecological concerns, including soil and water contamination, and negative effects on non-target organisms. As a result, there is a growing need for sustainable pesticides and alternative strategies to manage plant diseases more responsibly. Natural products, particularly

essential oils and plant-derived compounds, offer a promising solution due to their biodegradability, low toxicity, and broad-spectrum antimicrobial activities. These alternatives are not only effective against a range of plant pathogens but also align with integrated pest management (IPM) approaches that prioritize ecological balance and long-term sustainability in agriculture **(Isman, 2020).**

Medicinal and aromatic plants hold great significance primarily due to their content of plant secondary metabolites. These include essential oils, alkaloids, glycosides, saponins, tannins, vitamins, and various other bioactive compounds. Unlike primary metabolites, which are directly involved in fundamental physiological processes such as photosynthesis and respiration that contribute to the growth and development of plants, secondary metabolites serve different functions. They play essential ecological and physiological roles, often related to plant defense mechanisms against pathogens, herbivores, and environmental stress. These compounds are also responsible for the pharmacological and therapeutic effects that make medicinal and aromatic plants valuable in both traditional healing systems and modern pharmaceutical research **(Zheljazkov & Craker (2016)** *in* Zheljazkov & Cantrell, 2016**)**.

Essential oils are volatile, natural, complex compounds, produced by aromatic plants, predominantly found in temperate to tropical regions such as the Mediterranean, where they hold a significant place in traditional pharmacopoeia. They are characterized by a strong odour and are formed by aromatic plants as secondary metabolites. They are usually obtained by steam or hydro-distillation first developed in the Middle Ages by Arabs, play a vital role in nature as part of plant defense mechanisms, exhibiting antibacterial, antiviral, antifungal, insecticidal, and anti-herbivore activities.. Essential oils are typically liquid, clear, sometimes slightly colored, lipid-soluble, and miscible with organic solvents, with a density generally lower than that of water. They can be synthesized in various plant organs including buds, flowers, leaves, stems, seeds, fruits, roots, and bark, and are stored in specialized structures such as secretory cells, glandular trichomes, or epidermal tissues **(Bakkali et *al*., 2008).**

According to **Benarba & Meddah (2020),** Algeria's diverse ecosystems, ranging from Mediterranean coastlines to Saharan deserts, contribute to its rich biodiversity, making it a significant reservoir of medicinal and aromatic plants. The country's varied climates and terrains

support a wide array of flora, many of which have been traditionally used in folk medicine. Notably, species such as *Pistacia lentiscus* (mastic tree), *Lavandula* spp. (lavender), and *Ruta* spp. (rue) are indigenous to Algeria and have been traditionally utilized for their therapeutic properties.

Pistacia lentiscus **(Fig. 5)**, commonly known as the mastic tree, is a Mediterranean shrub renowned for its rich phytochemical profile and notable antimicrobial properties. Recent studies have highlighted that methanolic extracts from various parts of the plant, particularly the leaves, are abundant in phenolic acids and flavonoids. These compounds contribute significantly to the plant's biological activities. High-performance liquid chromatography (HPLC) analyses have revealed that leaf extracts possess higher concentrations of these bioactive compounds compared to stem and root extracts. In terms of antimicrobial efficacy, methanolic extracts have demonstrated superior activity over aqueous extracts, exhibiting inhibitory effects against a broad spectrum of bacterial strains, including both Gram-positive and Gram-negative bacteria. Moreover, these extracts have shown pronounced antifungal activity against species such as *Aspergillus niger*, *Aspergillus flavus*, and the yeast *Candida albicans*. The observed antimicrobial and antifungal activities underscore the potential of *Pistacia lentiscus* as a natural source for developing therapeutic agents and food preservatives **(Al-Zaben & *al.*, 2023).**

Figure 5 : The mastic tree *« Pistacia lentiscus »* **[7].**

Lavender (*Lavandula* spp.), a prominent member of the Lamiaceae family, is renowned for its aromatic properties and diverse applications in traditional medicine, cosmetics, and agriculture. The essential oils extracted from lavender, are rich in bioactive compounds such as linalool and linalyl acetate, which contribute to their notable antimicrobial activities **(Cavanagh & Wilkinson, 2005).** Lavender essential oils have long been utilized in complementary medicine, cosmetic products, and as food additives due to their traditional reputation for possessing strong antibacterial and antifungal properties **(Park & *al*., 2019).**

Lavandula stoechas **(Fig. 6)**, commonly known as French lavender, is a Mediterranean aromatic plant known for its essential oil, which exhibits various biological activities, including antifungal properties. The essential oil of *L. stoechas* is rich in compounds such as camphor, fenchone, and 1,8-cineole, which contribute to its antimicrobial efficacy. Recent studies have shown that L. stoechas essential oil demonstrates significant inhibitory effects against phytopathogenic fungi such as *Fusarium* spp., *Botrytis cinerea*, *Septoria* spp., and *Aspergillus niger*. Its broad-spectrum antifungal activity suggests its potential use as a natural alternative to synthetic fungicides in agricultural and postharvest applications **(El Abdali & *al*., 2022).**

Figure 6 : *Lavandula stoechas* **[8].**

Ruta, commonly known as rue, is a genus of aromatic, evergreen subshrubs in the Rutaceae family, native to the Mediterranean region, Macaronesia, and southwest Asia. The

most well-known species is *Ruta graveolens*, also known as common rue **(Fig. 7)**. These plants are characterized by their bluish-green, feathery leaves and yellow flowers. They are commonly cultivated in herb gardens worldwide for their medicinal properties and tolerance to hot and dry soil conditions. Traditionally, *Ruta* species have been used to treat various ailments, including digestive disorders, menstrual disturbances, and as an abortifacient **(Kannan & Babu, 2012)**.

Figure 7 : Rue « ***Ruta graveolens*** » **[9].**

Previous studies have highlighted that *Ruta officinalis* (*Ruta graveolens),* is a rich source of bioactive constituents, including alkaloids, flavonoids, furanocoumarins, and essential oils. These secondary metabolites are responsible for a wide range of biological properties, such as antimicrobial, antifungal, antioxidant, and anti-inflammatory activities. The essential oil of *R. officinalis* has particularly shown promising inhibitory effects against various pathogenic microorganisms, making it a potential natural agent for pharmaceutical and agricultural applications **(Benmansour & *al*., 2016).**

Kannan & Babu (2012) reported that, the genus *Ruta* has 14 accepted species, and among them *R. graveolens* L. and *R. chalepensis* L. . *Ruta graveolens* is cultivated as a medicinal and ornamental herb in many countries. The ancient Greeks and Romans, held the plant in high esteem. It is a well known remedy for the treatment of various types of disorders as reported in various classical texts of Ayurveda, Homoeopathy and Unani. More than 120 natural

compounds mainly including acridone, alkaloids, coumarins, essential oils, flavonoides, and fluoroquinolones have been found in the roots and aerial parts and various traditional uses and pharmacological properties are reviewed.

The exploration of essential oils derived from Mediterranean plants as natural pesticides has gained significant attention due to their potent antimicrobial and antifungal properties. This study investigates the yield and antifungal activity of essential oils and hydrosols extracted from the aerial parts of three aromatic plants, *Pistacia lentiscus*, *Lavandula stoechas*, and *Ruta graveolens* grown in the Guelma region of northeastern Algeria. The essential oils and hydrosols were tested against four plant pathogenic fungi: *Zymoseptoria tritici*, *Botrytis cinerea*, *Aspergillus niger* and *Fusarium graminearum.* The aim is to explore the potential of these natural products as botanical pesticides, offering an environmentally friendly alternative to synthetic chemicals by minimizing their harmful effects on non-target organisms and human health.

Material and methods

Chapter 1 : Material and methods

This study aims to assess the yields and antifungal properties of essential oils extracted from selected medicinal and aromatic plants grown in the Guelma region, located in Northeast Algeria by the determination of :

- ❖ Essential oil's (EOs) yield.
- ❖ EOs and Hydrosols characteristics.
- ❖ Antifungal activity of EOs and Hydrosols of the studied plants on plant pathogenic fungi.

1.1. Plant material

Our work focused on the aerial parts (leaves, fruits and flowers) of three species of aromatic and medicinal plants **(Tab. 1)** :

- ✓ The mastic tree *(Pistacia lentiscus*).
- ✓ Lavender (*Lavandula stoechas*).
- ✓ Rue (*Ruta graveolens*).

Table 1: The origin and characters of plants used.

Species	Family	Location	Date of collection	Parts used
Pistacia lentiscus	Anacardiaceae	Houara (Guelma) Altitude 36°53'21" North Longitude 7°58'41" East	December 2024	Fruits
Lavandula stoechas	Lamiaceae	Maouna (Guelma) Altitude 36°22'07 "North Longitude7°23'30.0"East	May 2024	Leaves and floral summits
Ruta graveolens	Rutaceae	Guelma (University) Altitude 36°45'07 "North Longitude7°41'34.0"East	April 2025	Leaves

The plant species were chosen based on several criteria:

- Their longstanding use in traditional medicine, particularly in the preparation of herbal teas and other products for treating microbial diseases and infections in humans.
- Their abundant availability as natural botanical resources throughout the country, with a focus on eastern Algeria.
- The limited research available on the biopesticidal properties of their essential oils, especially regarding their antifungal activity.

1.1.1. Presentation of Guelma region, origin of plant material

Guelma is located in the northeastern region of Algeria at coordinates 36°27'43"N and 7°25'33"E, with an elevation of approximately 305 meters above sea level. The region is characterized by a diverse topography that includes significant forest coverage and is traversed by the Seybouse River, the main hydrological feature in the area.The climate varies across the Wilaya: the central and northern areas experience a sub-humid Mediterranean climate, while the southern parts are classified as semi-arid. Winters are generally mild and rainy, whereas summers tend to be warm. Average temperatures range from 4°C in colder months to 35°C in the hottest periods, with extremes rarely dipping below 0°C or exceeding 39°C **[10].**

1.1.2. Samples treatment

After collection, the plants were carefully cleaned and air-dried in a well-ventilated area at room temperature for a period ranging from 7 to 20 days, depending on the species. Once fully dried, the relevant parts were separated from the rest of the plant, and stored in clean bags until further use.

1.1.3. Extraction of essential oils

Essential oils (EOs) were extracted by hydrodistillation using a *Clevenger* type apparatus **(Fig. 8)** at the laboratories of the Faculty of Natural and Life Sciences and Earth and Universe Sciences of 8th May 1945 University of Guelma.

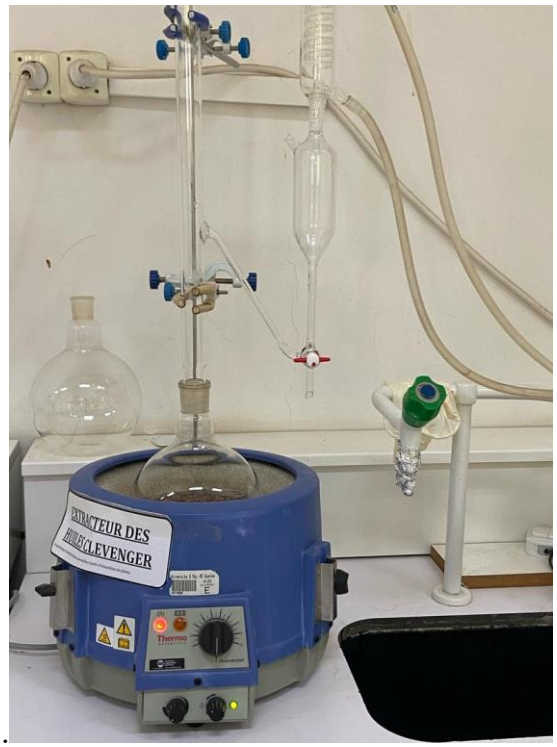

Figure 8: *Clevenger* apparatus used for essential oils extraction

Hydrodistillation is a classical technique employed for extracting essential oils from plant materials, particularly those sensitive to direct steam. In this method, plant material is fully immersed in water, which is then heated to produce steam. The steam carries the volatile compounds from the plant, which are subsequently condensed and separated to yield the essential oil. This process is advantageous for delicate flowers, such as rose and neroli, as it prevents potential damage that might occur with direct steam distillation **(Katekar & *al.*, 2023).**

The method results in two layers - an aqueous layer and an oil-rich layer. The oil can then be further separated **(Fagbemi & *al.*, 2021).** The aqueous layer is concerned as « Hydrosol » in this study.

1.1.4. Preservation of the essential oils obtained and hydrosols

The essential oils and hydrosols extracted from the various plant species used in this study were collected separately in clean, sterile containers. Essential oils were stored in a cool, dark place (congener), while hydrosols were kept in a freezer to preserve their properties.

1.1.5. Determination of yield's essential oils

Essential oil yield represents the amount of oil obtained from a specific quantity of dried plant material. It is usually expressed as a percentage and determined using the following formula:

$$YEO\ (\%) = (Mass\ of\ extracted\ essential\ oil\ /\ Mass\ of\ dried\ plant\ material) \times 100$$

YEO (%) : Essential oil yield (in percentage).

1.2. Fungal material

The study is focused on four plant pathogenic fungi species:

- *Zymoseptoria tritici,* causal agent of Septoria tritici bloch of wheat (STB)
- *Botrytis cinerea,* causal agent of gray mold on tomato and many vegetables.
- *Aspergillus niger,* causal agent of black mold on onion.
- *Fusarium graminearum,* wich affects wheat plants

1.2.1. Origins of fungi's samples

Zymoseptoria tritici was isolated from common wheat leaves exhibiting symptoms of Septoria tritici blotch, while *Botrytis cinerea* was obtained from infected tomato showing signs of gray mold. *Aspergillus niger* and *Fusarium graminearum* had been previously isolated from contaminated plant's organs by Dr. Allioui N. and is maintained as part of the laboratory's microbial collection.

1.2.2. Cultivation and preservation of strains

Fungal strains were grown on the nutrient medium PDA (Potato Dextrose Agar), incubated for 07 days at 24°C, and subsequently kept at 4°C.

1.3. Antifungal activity tests

1.3.1. Material and products used

The table **2** indicates the list of material and products used for fhe antifungal activity tests.

Table 2 : List of material and products used for fhe antifungal activity tests.

N°	Material	Products
1	Incubator, set at 24 °C.	Fungal strains.
2	Micro pipettes (2-20, 20-200 and 500 µL) + tips.	Physiological water solution.
3	Optical microscope.	Ethanol.
4	Bunsen burner.	Distilled water.
5	Parafilm.	Tween 80.
6	Hot plate.	Samples of EOs and Hydrosols.
7	Conical tube.	Culture media : PDA.
8	Platinium loop.	Sterilized distilled water.
9	Malassez cell.	
10	Precision balance.	
11	Vortex.	
12	Wattman paper discs (6 mm of diameter).	
13	Pasteur Pipettes.	
14	Petri dishes (9 cm of diameter).	

1.3.2. Preparation of spore suspensions

Spore suspensions of the various fungal strains were prepared from 7 day old cultures. Spores were collected by gently scraping them into 15 mL sterile plastic conical tubes containing a 0.9 % physiological saline solution supplemented with two drops of Tween 80. The spore concentrations were then adjusted according to values reported in the literature for each of the studied pathogens.

– For *Zymoseptoria tritici* : a sporal concentration of 3×10^6 spores/ mL **(Perelló & *al*., 2013).**

– For *Botrytis cinerea* : a sporal concentration of 10^6 spores/mL **(Lian & *al*., 2017).**

– For *Aspergillus niger* : a sporal concentration of 10^6 spores/mL **(Petrikkou & *al*., 2001).**

– For *Fusarium graminearum :* a sporal concentration of 10^5 spores/mL **(Remmal & *al*., 2014).**

1.3.3. Fungal Strains-Essential Oils Susceptibility test

For testing the susceptibility of the studied fungal strains to essential oils, four volumes of pure essential oils were used: 2.5 µL, 5 µL, 10 µL, 20 µL, and, along with a negative control (0 µL) treated with sterilized distilled water.

The antifungal activity of essential oils (EOs) was evaluated using the disk diffusion method. This direct confrontation technique involves the diffusion of EOs from 6 mm diameter Wattman paper discs placed at the center of Petri dishes containing potato dextrose agar (PDA) previously inoculated with the fungal strains **(Khalili *& al.*, 2012**). A 500 µL spore suspension, freshly prepared on the same day from an active culture, was evenly spread over the surface of the PDA in each 90 mm Petri dish. After allowing the suspension to dry for a few minutes, a 6 mm Whatman paper disc was positioned in the center of each dish and impregnated with the selected volume of EO. The inoculated plates were then incubated at 24°C.

Fungal growth inhibition was assessed by visually examining and measuring the diameter of the clear zones around the EO-treated discs after 72 hours of incubation. Each EO volume was tested in triplicate to ensure reproducibility of the results.

1.3.4. Fungal Strains-Hydrosol Susceptibility test

To assess the susceptibility of the fungal strain to hydrosol, three volumes of pure hydrosol were tested: 25 µL, 50 µL, and 100 µL, along with a negative control (0 µL) treated with sterilized distilled water. Each volume was tested in triplicate to ensure consistency.

'Well diffusion' method (WD) it's about using a 90 mm diameter Petri dishes containing solidified PDA medium, a central well was created using a sterile Pasteur pipette. This well was then filled with the selected volume of hydrosol (**Magaldi & *al.,* 2004**). The plates were subsequently incubated at 24°C, and the results were recorded after 72 hours of incubation.

1.4. Data treatment and analysis

Microsoft Excel 2010 was used to process and analyze the obtained results, including calculating average inhibition zone diameters, standard deviations and figures. The Minitab software, version 2018, was used to perform an analysis of variance and Dunnett test on the results obtained for the various strains and treatments.

Results and discussion

Chapter 2 : Results and discussion

2.1. Yields of Essential oils (EO)

The essential oil yields from the studied plants, as shown in **Figure 9**, indicate that *Lavandula stoechas* produced the highest yield (0.872 ± 0.113%), followed by *Ruta graveolens* with a yield of 0.297 ± 0.073%. In contrast, *Pistacia lentiscus* showed the lowest yield, measuring 0.073 ± 0.017%.

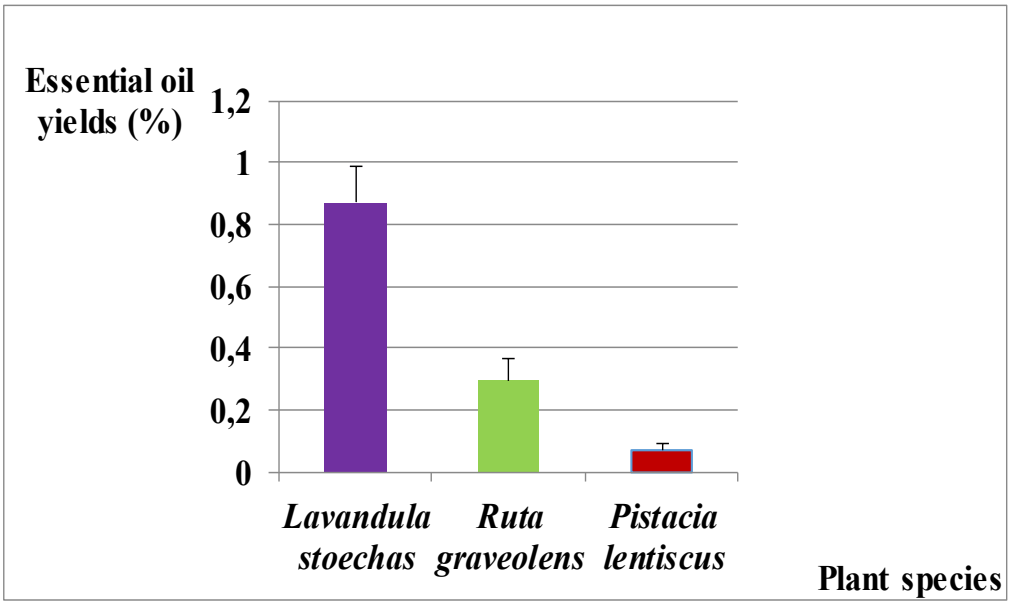

Figure 9 : Yields of the essential oils of plants used in this study.

Comparing our results with those reported in the literature, we note that :

- **For *Lavandula stoechas*:**

  The extracted yield of the essential oil from *Lavandula stoechas*'s leaves and flowers is 0.872 ± 0.113%. This is considered nearly similar to the yield obtained by **Bouadjina & Louati (2024)** for the same specie collected from Bouati mahmoud (W. Guelma, Algeria) in April. They have recorded a yield of 0.882 ± 0.12%. **Zuzarte & *al.* (2013)**, have obtained a yield of 0.7 % from aerial parts of the same specie, collected from Sardinia (Italy), and using the same extraction method.

On the other hand, our values (0.872 ± 0.113%) are lower than those obtained by **Özcan & *al.* (2018),** using flowers of the same plant, from Konia provence (Turkey), They obtained a yield of 2.9 %, but it is more important compared to that obtained by **Alami & *al.* (2016)** using the leaves of the same plant, collected from two sites in Morocco, by hydrodistillation. The authors achieved yields of 0.12 % and 0.45 %.

Angioni & *al.* (2006), have obtained a yield of 2.7 % (2004) and 3.1 % (2005) from flowers of *Lavandula stoechas* collected in April from southern Sardinia (Italy), extracted by hydrodistillation. The authors noticed a great seasonal variability in, yield, chemical composition, and antifungal activity of *Lavandula stoechas L. ssp. stoechas* essential oils from stem/leaves and flowers.

- **For *Ruta graveolens*:**

The obtained yield of the essential oil from *Ruta graveolens*'s leaves (0.297 ± 0.073%), is considered similar to that obtained by **Attia & *al.* (2018),** the authors have recorded a yields of EOs of 0.366 and 0.215% v/w for leaves and flowers, respectively.

Jaradat (2016) have compared two mains of extraction methods (Hydrodistillation and microwave accelered distillation method), of two *Ruta* species (*R. graveolens* and *R. chalepensis*), collected from Palestine, and has obtained an important yield of essential oil compared to our results. The recorded values were, 2.2% yield in *Ruta graveolens* and approximately 1.8% yield of the volatile oils in *Ruta chalepensis* on a dry weight basis, by microwave accelered distillation method, and in the same time the yield was approximately 1.2% in *Ruta graveolens* while was 0.6% yield of the volatile oils in *Ruta chalepensis* by using hydrodistillation method.

In this previous study, the author has concluded that the isolation of the volatile oils from *R. graveolens* and *R. chalepensis* by microwave assisted extraction is more efficient in time cost and yield rather than hydrodistillation ; also the results showed that the best economical source for Rue oil is *R. graveolens* for food, cosmeceuticals and pharmaceutical industries.

- **For *Pistacia lentiscus* :**

The average yield obtained on the basis of three successive extractions was 0.073 ± 0.017%. Essential oil yield obtained for this specie collected from Guelma in 2024, is less than those reported for the same specie collected from Oran by **Mizi & *al*. (2021)** which is 1.26%.

Gardeli & *al.* (2008) have obtained variable essencial oil yield in leaves of *Pistacia lentiscus* collected from a Greek island according to the stage plant. They have recorded 0.30 % in samples collected before flowering (February) and flowering (May), and 0.28 % in fruiting stage (August).

A value of 0.1 9 ±0.08 % have recorded by **Hajji-Hedfi & *al.* (2024),** from leaves of *P. lentiscus,* collected from the Kairouan region in the Center East of Tunisia.

Dlih Boudiaf *& al.* (2021) have obtained a yield of 0.0027 ml/100g, from aerial parts of *P. lentiscus* collected from Collo (W. Skikda, Algeria) on May-September 2018.

An oil yield of 0.14 % was obtained by **Amhamdi & *al*., (2009),** from leaves collected from eastern Morocco. The oil yield of *P. lentiscus* L. seems to depend on the nature of parts of plants used for extraction and also on the mode of extraction.

Variance analysis (**Tab. 3**) has shown very highly significant differences between oil's yields from the different species.

Table 3 : Results of variance analysis of EO's yields

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Yields	2	1,01852	0,509260	55,86	0,000 ***
Error	6	0,05470	0,009116		
Total	8	1,07322			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference

2.2. Organoleptic properties of the essential oils

The quality of an essential oil (EO) is influenced by several factors, including its appearance, color, and scent. These organoleptic properties notably, those detected through the senses, serve as important indicators of the oil's overall quality. Characteristics of the oils obtained in this study are indicated in table 4.

Table 4 : Organoleptic properties of essential oils extracted in this study

Origin of EOs	Color	Smell	Aspect
Lavandula stoechas	yellowish	Strong smell	Liquid
Ruta graveolens	Light yellow	Fresh strong smell	Liquid
Pistacia lentiscus	Pale yellow	Strong smell	Liquid

Gardeli & *al*., (2008) have obtained a yellow essential oil for *P. lentiscus* L. **Dlih Boudiaf *& al.* (2021),** have obtained yellow oil with a characteristic pungent odour, from aerial parts of *P. lentiscus* collected from Collo (W. Skikda, Algeria) on May- September 2018.

A dark -yellow color, liquid and limpid aspect, strong, spicy favor smell, a pH of 4.29 ± 0.011, a density of 0.877±0.005 and acid index of 1.87 ± 0.005, have obtained by **Hajji-Hedfi & *al.* (2024)** from leaves of this specie, collected from the Kairouan region in the Center East of Tunisia.

Amara & *al*. (2019) have obtained the same characteristics for *Pistacia lentiscus* essentiel oil extracted from fruits, collected in november from Tipaza (Algeria), and they confirmed that are similar to those described by AFNOR.

2.3. Antifungal activity results

2.3.1. Antifungal activity of EOs

2.3.1.1. Effects of essential oils on the growth of *Aspergillus niger*

Figure 10 presents the effects of the different EOs on the growth of *Aspergillus niger.*

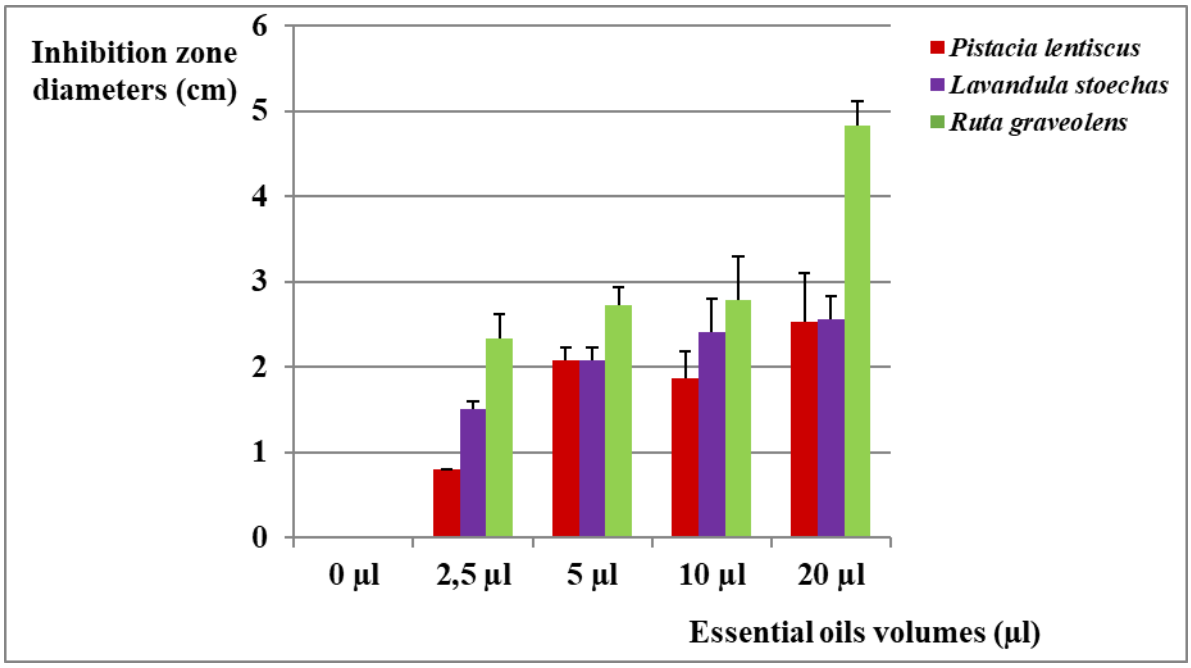

Figure 10 : Diameters of growth inhibition zones of *Aspergillus niger* versus the three essential oils (*P. lentiscus*, *L. stoechas* and *R. graveolens*).

Comparing the average inhibition zone values for *Aspergillus niger*, we can see, that, at 20 µL EO of *R. graveolens* has the highest average inhibition zone with a value of 4.833 ± 0.288 cm. *L. stoechas* has the second-highest average inhibition zone with 2.566 ± 0.273 cm, while *P. lentiscus* has the lowest average inhibition zone with 2.533 ± 0.5773 cm.

When considering the recorded values for *Aspergillus niger*, *R. graveolens* have higher inhibition zone diameter comparing to *P. lentiscus* and *L. stoechas. At all tested volumes.*

Kannan & Babu (2012) reported that, *Ruta graveolens* is rich in bioactives molecules, and more than 120 natural compounds mainly including acridone, alkaloids, coumarins, essential

oils, flavonoides, and fluoroquinolones have been found in the roots and aerial parts, and various traditional uses and pharmacological properties are reviewed for this specie.

Kengar and Paratkar (2014), have analyzed the methanolic extract and oil of leaves of *R. graveloens* L. for antifungal activity against the pathogenic fungal strains of *Candida albicans*, *Cryptococcus neoformans,* and *Aspergillus niger,* using agar well method. The degree of antifungal activity was measured in terms of zone of inhibition. Methanolic extract showed varying degrees of antifungal activity on the tested fungal strains. It was found that *A. niger* was more susceptible than *C. albicans*. The Methanolic extract at higher concentration of 100µl showed 26 mm zone of inhibition in *A. niger.* However, in case of essential oil, the *C. albicans* was more susceptible than the other two fungal species. The maximum zone of inhibition (26 mm) was recorded in *C. albicans* at higher concentration of oil (100 µl). The research findings supports the traditional usage of *R. graveolens* L. leaves extract and essential oils which possess compounds with antifungal properties that can be used for development in new useful drugs and compounds as antifungal agents for the treating infectious diseases.

Kengar and Paratkar (2014), have tested the antifungal activity of the essential oil of *R. graveolens* L. on *Aspergillus niger* by the agar well diffusion method, and they have recorded an inhibition zone of 15 mm at 100 µl/ ml. Also, the autors have reported that, the ethyl acetate extract of *Ruta graveolens* leaves, shown fungicidal activity against several agriculturally important pathogenic fungi like *Colletotichum fragariae*, *C. gloeosporioides, C. acutatum*, *Botrytis cineara* and *Fusarium oxysporium*.

Mezni & *al.*, (2015) have evaluate the antifungal activity of *Pistacia lentiscus* extracts against *Aspergillus niger* and have shown an inhibition zones of 9.33 mm.

In a study assessing the antimicrobial activity of *Lavandula officinalis* (closely related to *L. stoechas*) essential oil against various pathogens, including *Aspergillus niger*, it was found that the oil exhibited high antimicrobial activity against *Aspergillus niger* with an inhibition zones of 10.7 mm (**Maghnia, 2023).**

Variance analysis has shown very high significant differences between volumes for *Aspergillus niger* versus *L. stoechas* EO (**Tab. 5**), *Aspergillus niger* versus *P. lentiscus* EO (**Tab. 6**) and *Aspergillus niger* versus *R. graveolens* EO (**Tab. 7**).

Table 5: Results of variance analysis of *Aspergillus niger* versus *L. stoechas* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	13,0223	3,25558	64,47	0,000***
Error	10	0,5050	0,05050		
Total	14	13,5273			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Table 6 : Results of variance analysis of *Aspergillus niger* versus *P. lentiscus* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	11,157	2,7893	26,31	0,000 ***
Error	10	1,060	0,1060		
Total	14	12,217			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Table 7: Results of variance analysis of *Aspergillus niger* versus *R. graveolens* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	41,5177	10,3794	108,50	0,000***
Error	10	0,9567	0,0957		
Total	14	42,4743			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Dunnett tests have also shown very high significant differences between control and tested volumes for *Aspergillus niger* versus the Eos of the three plant species (**Tab.8**).

Table 8: Results of Dunnett tests of *Aspergillus niger* versus the three EO

Volume of EO / Plants species	0µl	2,5µl	5µl	10µl	20µl
Lavandula stoechas **(Means ± σ)**	0 ± 0	1,5 ± 0,1 ***	2,083 ± 0,144 ***	2,416 ± 0,381 ***	2,566 ± 0,275 ***
Pistacia lentiscus **(Means ± σ)**	0 ± 0	0,8 ±1,359 ***	2,083± 0,144 ***	1,866 ±0,321 ***	2,533± 0,577 ***
Ruta graveolens **(Means ± σ)**	0 ± 0	2,333 ± 0,288 ***	2,783 ± 0,202 ***	4,083 ± 0,520 ***	4,833 ± 0,288 ***

*** : Very highly significant differences (α=0,001)

2.3.1.2. Effect of essential oils on the growth of *Botrytis cinerea*

According to the results obtained in this study **(Fig. 11)** all oils tested have inhibited the growth of *Botrytis cinerea*. *L. stoechas* and *R. graveolens* seem to have an important effect on the growth of the fungus with all volumes. At 20 µL, a complete growth inhibition was observed (an inhibition zone diameter of 9 ± 0 cm) for *R. graveolens* and *L. stoechas,* and moderate effect was observed for *P. lentiscus*, against this fungus (3.316 ± 0.160 cm).

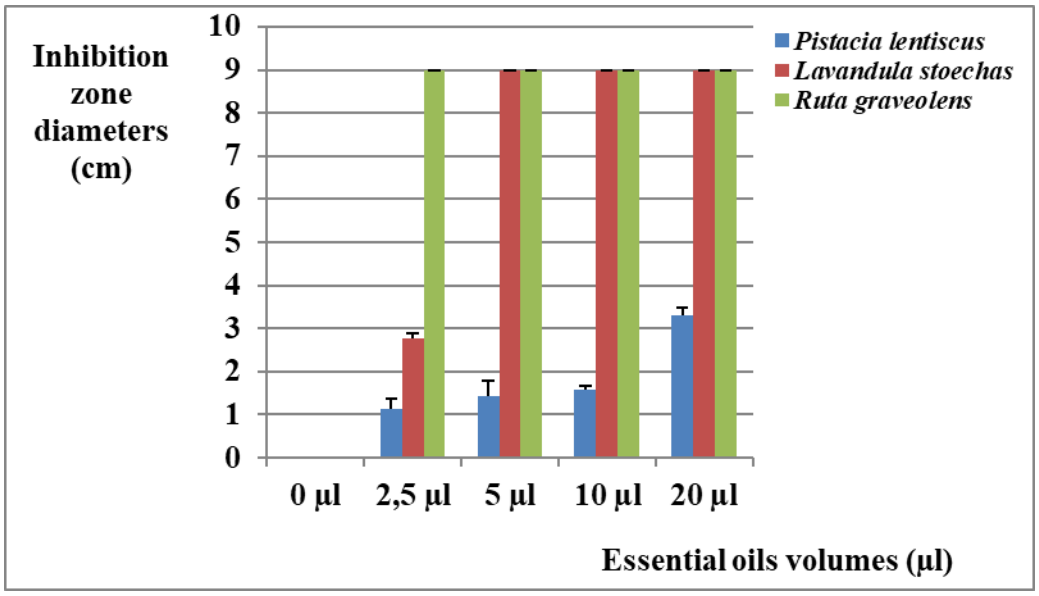

Figure 11 : Diameters of growth inhibition zones of *Botrytis cinerea* versus the three essential oils (*P. lentiscus*, *L. stoechas* and *R. graveolens*).

Soylu & *al.* (2010) have evaluated the antifungal activity of *Lavandula stoechas* essential oil against *Botrytis cinerea* and have found satistactory results.

The inhibitory effects of essential oils of flowers of lavender (*L. stoechas*) were determined against *A. alternata, F. oxysporum* and *B. cinerea*. 40 ppm dose of oil caused a complete inhibition (100%) against mycelial growth of *B. cinerea*. The analysis showed that lavender oil exhibited fungistatic activity at different levels depending on the doses (**Özcan & *al*., 2018).**

A study investigated the antifungal activity of *Ruta graveolens* essential oil against *Botrytis cinerea* have demonstrate, that, the essential oil was also found to be active against *Botrytis cinerea,* with a minimum inhibitory concentration (MIC) of 100–1100 µg/mL (**Pereira & *al*., 2021).**

Allagui & *al*. (2024), have tested thirty EOs at 0.5 mg/mL, for in vitro growth inhibition of the main postharvest fungi, which are *Alternaria alternata*, *Botrytis cinerea*, and *Penicillium italicum*. They concluded that *B. cinerea* displayed the highest sensitivity to Eos than *P. italicum* and *A. alternata*, and *B. cinerea*, mycelial growth was completely inhibited by *Cinnamomum verrum, Gautheria fragrantissima*, *Cymbopogon nardus*, *Pelargonium asperum*, and *Cupressus sempervirens* EOs.

Variance analysis has shown very high significant differences between volumes for *Botrytis cinerea* versus *L. stoechas* EO (**Tab. 9**), *Botrytis cinerea* versus *P. lentiscus* EO (**Tab. 10**) and significant difference between volumes for *Botrytis cinerea* versus *R. graveolens* EO (**Tab. 11**).

Table 9: Results of variance analysis of *Botrytis cinerea* versus *L. stoechas* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	220,331	55,0827	20656,00	0,000***
Error	10	0,027	0,0027		
Total	14	220,357			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Table 10: Results of variance analysis of *Botrytis cinerea* versus *P. lentiscus* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	17,0043	4,25107	109,73	0,000***
Error	10	0,3874	0,03874		
Total	14	17,3917			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Table 11: Results of variance analysis of *Botrytis cinerea* versus *R. graveolens*EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	194,400	48,600	*	*
Error	10	0,000	0,0000		
Total	14	194,400			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
* : significant difference**.**

Dunnett tests have also shown very high significant differences between control and tested volumes for *Botrytis cinerea* versus the Eos of the three plant species, except for 2.5 µL of *Pistacia lentiscus* where no significant differences were noticed (**Tab.12**).

Table 12: Results of Dunnett tests of *Botrytis cinerea* versus the three EO

Volume of EO / Plants species	**0µl**	**2,5µl**	**5µl**	**10µl**	**20µl**
Lavandula stoechas **(Means ± σ)**	0 ± 0	2,766 ± 0,115 ***	9 ± 0 ***	9 ± 0 ***	9 ± 0 ***
Pistacia lentiscus **(Means ± σ)**	0 ± 0	1,113 ± 0,230 **NS**	1,416 ± 0,378 ***	1,566 ± 0,115 ***	3,316 ± 0,160 ***
Ruta graveolens **(Means ± σ)**	0 ± 0	9 ± 0, ***	9 ± 0, ***	9 ± 0, ***	9 ± 0 ***

NS : No significant differences (α=0,05)
*** : Very highly significant differences (α=0,001)

2.3.1.3. Effect of essential oils on the growth of *Zymoseptoria tritici*

Figure 12 show the results obtained for this fungus versus tested Eos.

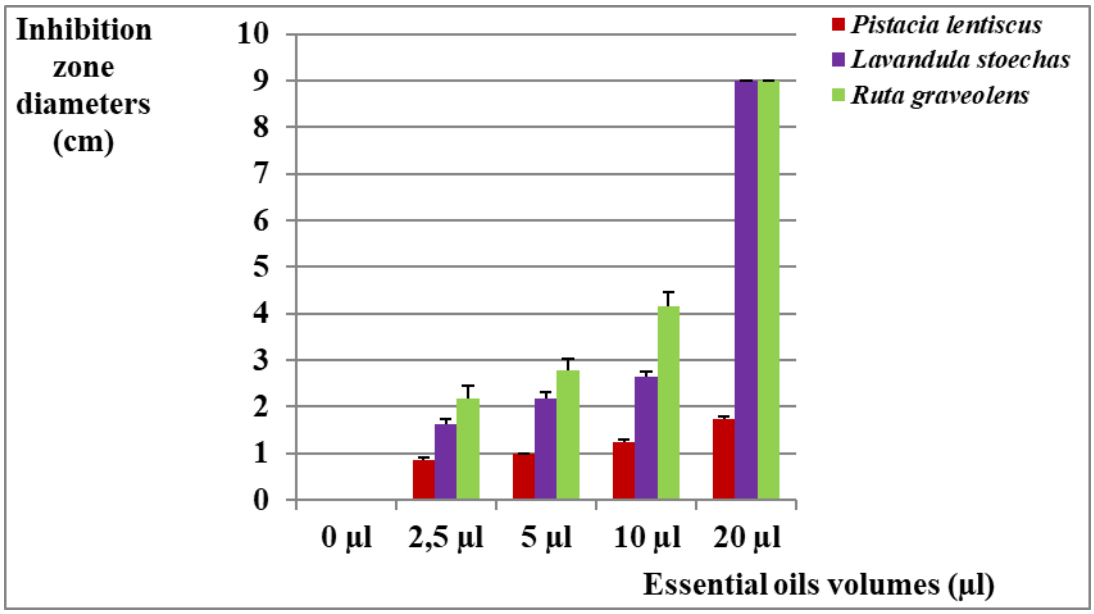

Figure 12 : Diameters of growth inhibition zones of *Zymoseptoria tritici* versus the three essential oils (*P. lentiscus*, *L. stoechas* and *R. graveolens*).

Comparing the average inhibition zone values for *Zymoseptoria tritici* **(Fig. 12)**, we can see, at 20 µL that EO of *R. graveolens* and, *L. stoechas* have the highest average inhibition zone with a value of 9 ± 0.000 cm. while *P. lentiscus* has the lowest average inhibition zone with 1.733 ± 0.0577 cm.

Bouadjina & Louati (2024), have evaluated the antifungal activity of *Lavandula stoechas* subsp. *pedunculata* essential oil against *Zymoseptoria tritici* and obtained an average inhibition zone at the volume of 40 µL with a value of 12.333 ± 0.577mm. At 5 µL, the inhibition zone diameter was 8.333 ± 0.577mm.

In the same study, the antifungal activity of *Ruta montana* (a specie closely related to *Ruta graveolens*) essential oil against *Zymoseptoria tritici* was assessed: Comparing the average inhibition zone values for *Zymoseptoria tritici*, we can observe that the volume of 40 µL of the essential oil has the highest average inhibition zone with a value of 14 ± 1 mm, and at 5 µL, the inhibition zone was 9.666 ± 0.577 mm.

Our study have shown an important effect of *R. graveolens* EO against *Z. tritici,* compared with *R. montana* EO used by **Bouadjina & Louati (2024)**, and we have recorded an antifungal activity at very low volumes, 2.5 µL (2,166 ± 0,288 cm), and at 20 µL we have noticed a complete growth inhibition of this fungus (9.00 ± 0,000 cm).

Variance analysis has shown very high significant differences between volumes for *Zymoseptoria tritici* versus *L. stoechas* EO (**Tab. 13**), *Zymoseptoria tritici* versus *P. lentiscus* EO (**Tab. 14**) and for *Zymoseptoria tritici* versus *R. graveolens* EO (**Tab. 15**).

Table 13: Results of variance analysis of *Zymoseptoria tritici* versus *L. stoechas* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	142,561	35,6402	2852,74	0,000***
Error	10	0,125	0,0125		
Total	14	142,686			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Table 14: Results of variance analysis of *Zymoseptoria tritici* versus *P. lentiscus* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	4,86933	1,21733	608,67	0,000***
Error	10	0,02000	0,00200		
Total	14	4,88933			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Table 15: Results of variance analysis of *Zymoseptoria tritici* versus *R. graveolens* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	135,479	33,8698	728,38	0,000 ***
Error	10	0,465	0,0465		
Total	14	135,944			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Dunnett tests have also shown very high significant differences between control and tested volumes for *Zymoseptoria tritici* versus the Eos of the three plant species (**Tab.16**).

Table 16: Results of Dunnett tests of *Zymoseptoria tritici* versus the three EO

Volume of EO / Plants species	0µl	2,5µl	5µl	10µl	20µl
Lavandula stoechas **(Means ± σ)**	0 ± 0	1,633 ± 0,115 ***	2,166 ± 0,152 ***	2,633 ± 0,115 ***	9 ± 0,0 ***
Pistacia lentiscus **(Means ± σ)**	0 ± 0	0,866 ±0,057 ***	1 ± 0 ***	1,233 ±0,057 ***	1,733 ± 0,057 ***
Ruta graveolens **(Means ± σ)**	0 ± 0	2,166 ± 0,288 ***	2,783 ± 0,256 ***	4,166 ± 0,288 ***	9 ± 0 ***

*** : Very highly significant differences (α=0,001)

2.3.1.4. Effect of essential oils on the growth of *Fusarium graminearum*

Figure 13 presents the effects of the different EOs on the growth of *Fusarium graminearum*

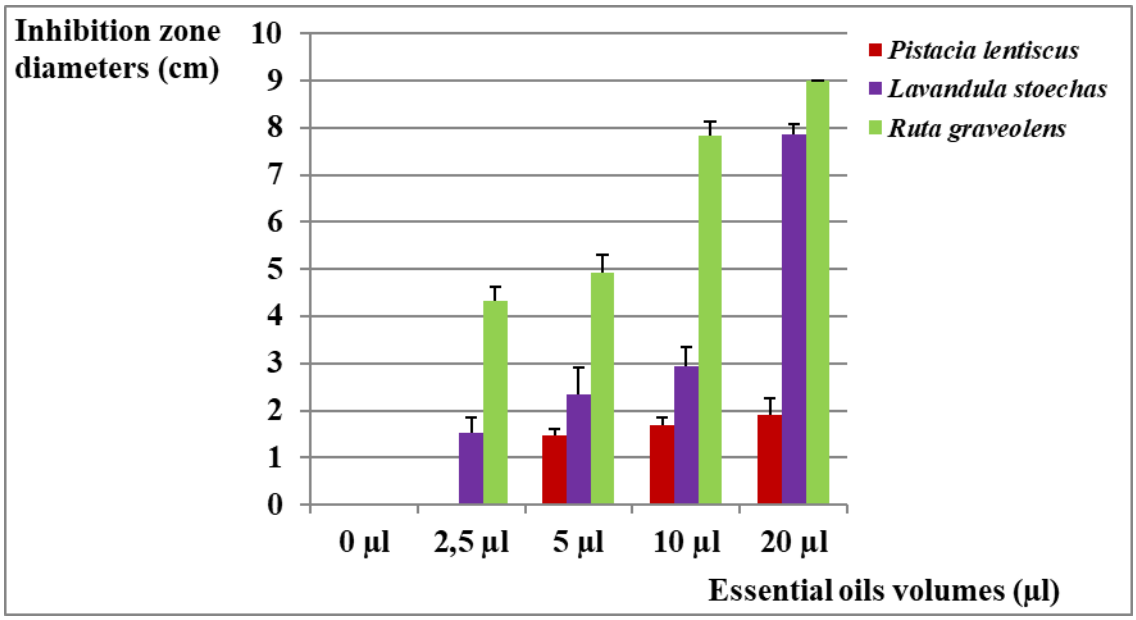

Figure 13 : Diameters of growth inhibition zones of *Fusarium graminearum* versus the three essential oils (*P. lentiscus*, *L. stoechas* and *R. graveolens*).

From **figure 13**, it can be seen that *R. graveolens* had the highest average antifungal activity against *Fusarium graminearum*, with a value of 9 ± 0.000 cm of zone inhibition diameter (Total growth inhibition) at 20 µL of EOs. *P. lentiscus* has recorded the lowest antifungal activity against this fungus.

Enayati & *al.* (2019), have evaluated the antifungal activity of *Lavandula stoechas* essential oil against *Fusarium graminearum* and found that the micelial growth inhibition of *L. stoechas* EO at 1000 µL/L concentration on *F. graminearum* was 66.7%. Specific data on the antifungal activity of *Ruta graveolens* essential oil against *Fusarium graminearum* is limited. However, a study on a related species, *Ruta chalepensis*, reported, that, at 200 µL/mL of *Ruta chalepensis* L. oil, zone inhibition efficacy against microbial strains was demonstrated (**Bouajaj & *al.*, 2014).**

Harcarova & *al.* (2021) have tested six essential oils (*Syzygium aromaticum*, *Origanum vulgare*, *Thymus vulgaris*, *Hyssopus officinalis*, *Ocimum basilicum*, *Myristica fragrans*) against two strains of *Fusarium graminearum*. According to the results obtained, the best antifungal activity against the both strains of *Fusarium graminearum* was demonstrated by *Origanum vulgare* EO at the concentration 100 µg/mL. They concludes, that, in the protection of plants against pathogenic fungi, essential oils appear to be a suitable substitute for synthetic chemicals. **Perczak & *al.* (2019)** have also demonstrated that essentiels oils, affected the growth of *Fusarium graminearum.*

Variance analysis has shown very high significant differences between volumes for *Fusarium graminearum* versus *L. stoechas* EO (**Tab. 17**), *Fusarium graminearum* versus *P. lentiscus* EO (**Tab. 18**) and for *Fusarium graminearum* versus *R. graveolens* EO (**Tab. 19**).

Table 17: Results of variance analysis of *Fusarium graminearum* versus *L. stoechas* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	105,787	26,4468	200,10	0,000***
Error	10	1,322	0,1322		
Total	14	107,109			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Table 18: Results of variance analysis of *Fusarium graminearum* versus *P. lentiscus* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	10,4110	2,60275	62,97	0,000***
Error	10	0,4133	0,04133		
Total	14	10,8243			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Table 19: Results of variance analysis of *Fusarium graminearum* versus *R. graveolens* EO

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	4	147,733	36,9333	590,93	0,000***
Error	10	0,625	0,0625		
Total	14	148,358			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Dunnett tests have shown very high significant differences between tested volumes and control for *Fusarium graminearum* versus the Eos of the three plant species exept for 2,5 µl volume of *P. lentiscus* where difference was no significant with the control (**Tab.20**).

Table 20: Results of Dunnett tests of *Fusarium graminearum* versus the threeEO

Volume of EO / Plants species	**0µl**	**2,5µl**	**5µl**	**10µl**	**20µl**
Lavandula stoechas **(Means ± σ)**	0 ± 0	1,533 ± 0,305 ***	2,333 ± 0,577 ***	2,933 ± 0,404 ***	7,866 ± 230 ***
Pistacia lentiscus **(Means ± σ)**	0 ± 0	0 ± 0 **NS**	1,466 ± 0,152 ***	1,7 ± 0,141 ***	1,9 ± 0,360 ***
Ruta graveolens **(Means ± σ)**	0 ± 0	4,333 ± 0,288 ***	4,916 ± 0,381 ***	7,833 ± 0,288 ***	9 ± 0 ***

NS : No significant difference (α=0,05)
*** : Very highly significant differences (α=0,001)

2.3.2. Antifungal activity of hydrosols (Hd)

2.3.2.1. Effects of hydrosols on the growth of *Aspergillus niger*

Figure 14 show that hydrosols collected after distillation of *R. graveolens* and *L. stoechas* have also affected growth of *Aspergillus niger*, especially for *L. stoechas* which an average of inhibition zone diameter of 2.333 ± 0.288 cm has been obtained at 100 µl of hydrosol. However, for hydrosol of *P. lentiscus*, no inhibition zone has been observed at all tested volumes of hydrosol except at 100 µl with a value of 0.7 ± 1.359 for this fungus.

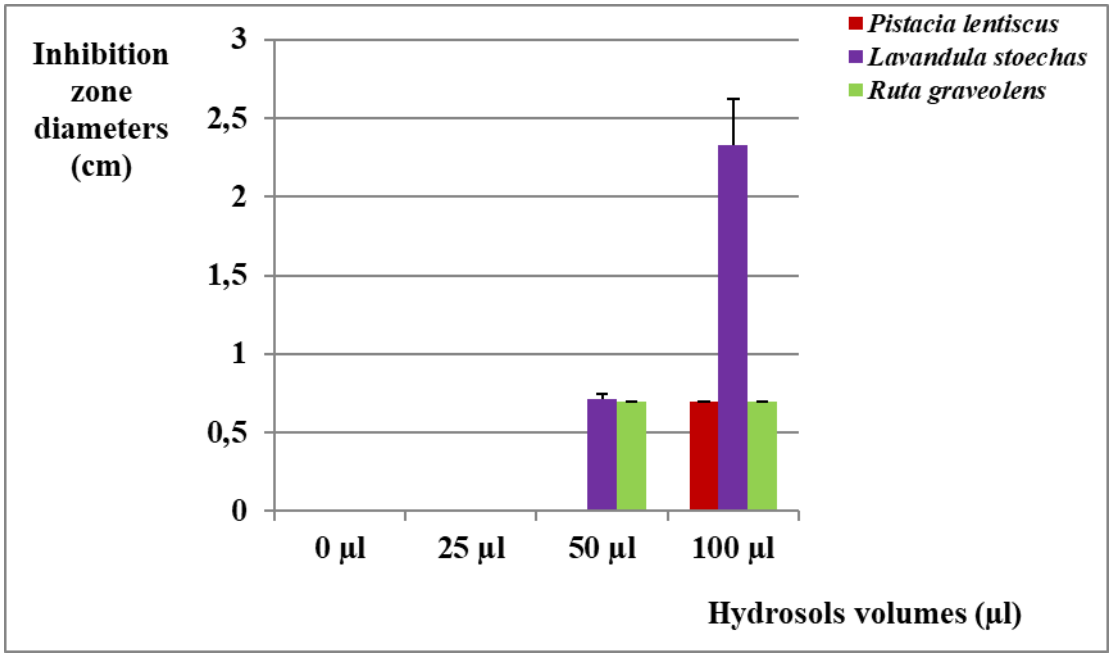

Figure 14 : Diameters of growth inhibition zones of *Aspergillus niger* versus the three hydrosols

Kengar and Paratkar (2014), have tested the antifungal activity of methanolic extract of *R. graveolens* L. on *Aspergillus niger* by the agar well diffusion method, and they have recorded an inhibition zone of 26 mm at 100 µl.

Pistacia lentiscus extracts showed greater inhibitory activity against the tested fungal strains *A. niger* and *A. flavus,* and yeast *C. albicans* (**Al-Zaben & *al.*, 2023).** According the authors, these extracts can be used to treat antimicrobial infections and as food preservatives.

Belabbes & *al.* (2017) have studied the chemical composition of the essential oils and hydrosol extract from aerial parts of *Calendula arvensis* L. The intra-species variations of the chemical compositions of essential oils from 18 Algerian sample locations were investigated using statistical analysis. The study of the chemical variability of essential oils allowed the discrimination of two main clusters confirming that there is a relation between the essential oil compositions and the harvest locations.

In another hand, the authors have used different concentrations of essential oil and hydrosol extract to test their antioxidant and antifungal activities, and the results showed that hydrosol extract presented an interesting antioxidant activity. The *in vitro* antifungal activity of hydrosol extract produced the best antifungal inhibition against *Penicillium expansum* and *Aspergillus niger*, while, essential oil was inhibitory at relatively higher concentrations. Results showed that the treatments of pear fruits with essential oil and hydrosol extract presented a very interesting protective activity on disease severity of pears caused by *P. expansum*.

Variance analysis has shown very high significant differences between tested volumes for *Aspergillus niger* versus *L. stoechas* Hydrosol (**Tab. 21**), high significant differences for *Aspergillus niger* versus *P. lentiscus* Hydrosol (**Tab. 22**) and no significant defferences for *Aspergillus niger* versus *R. graveolens* Hydrosol (**Tab. 23**).

Table 21: Results of variance analysis of *Aspergillus niger* versus *L. stoechas* Hydrosol

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	3	9,3890	3,12965	37,46	0,000***
Error	8	0,6683	0,08354		
Total	11	10,0573			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference**.**

Table 22: Results of variance analysis of *Aspergillus niger* versus *P. lentiscus* Hydrosol

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	3	1,0700	0,35667	9,95	0,004**
Error	8	0,2867	0,03583		
Total	11	1,3567			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F .
P : Likelihood of highlighting significant difference .
** $p<0,01$: highly significant difference .

Table 23: Results of variance analysis of *Aspergillus niger* versus *R. graveolens* Hydrosol

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	1,47000	0,490000		**NS**
Error	8	0,00000	0,00000		
Total	11	1,47000			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
NS : No significant difference ($\alpha=0,05$).

Dunnett tests have shown very high significant differences between 50 µl and 100 µl volumes and control for *Aspergillus niger* versus *L. stoechas* and *R. graveolens*, and 100 µl of *P. lentiscus* hydrosols, and no significant differences between control and all other volumes of hydrosols, of the all studied plant species (**Tab.24**).

Table 24: Results of Dunnett tests of *Aspergillus niger* versus the three hydrosols

Volume of HD / Plantsspecies	0µl	25µl	50µl	100µl
Lavandula stoechas **(Means ± σ)**	0 ± 0 **NS**	0 ± 0 **NS**	0,716 ± 0,028 ***	2,333 ± 0,288 ***
Pistacia lentiscus **(Means ± σ)**	0 ± 0 **NS**	0 ± 0 **NS**	0 ± 0 **NS**	0,7 ± 1,359 ***
Ruta graveolens **(Means ± σ)**	0 ± 0 **NS**	0 ± 0 **NS**	0,7 ± 1,359 ***	0,7 ± 1,359 ***

NS : No significant difference (α=0,05)
*** : Very highly significant differences (α=0,001)

2.3.2.2. Effect of hydrosols on the growth of *Botrytis cinerea*

From **figure 15**, it can be seen that *P. lentiscus* and *L. stoechas* had the highest average antifungal activity against *Botrytis cinerea*, with a value of 9 ± 0.000 cm of zone inhibition diameter at 50 µl and 100 µl of hydrosol. *R. graveolens* has recorded the lowerest antifungal activity at 100 µl with a value of 1.433 ± 0.1154 cm, and no activity was obtained in 25 µl and 100 µl for this plant against this pathogen.

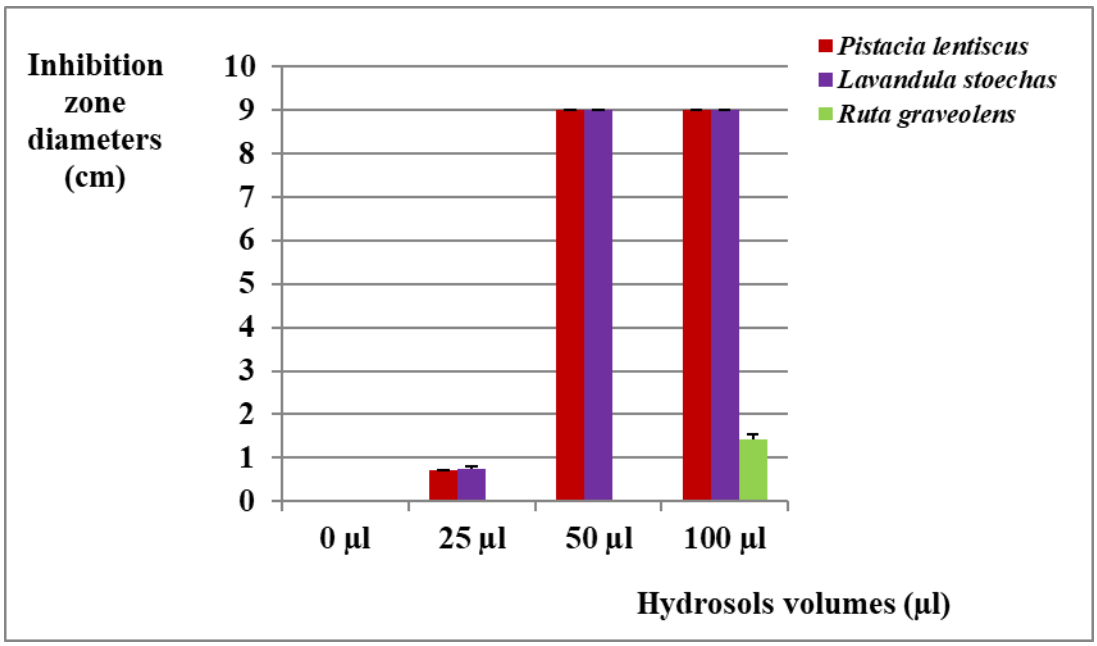

Figure 15 : Diameters of growth inhibition zones of *Botrytis cinerea* versus the three hydrosols

Necir and Amrani (2023) reported that, the efficacy of plant aqueous extracts is much lower than that of plant essential oils; however, extract preparation is much simpler. It was also found that hydrosol extract of *Daucus carota* subsp. *sativus* possess an in vitro antifungal activity against gray mould disease agent *B. cinerea*. In vivo fungicidal activity of hydrosol extracts of *D.carota* subsp. *sativus* was also investigated in post-harvest conditions. The results showed that hydrosol extract had a preventive effect of 70% up7 days of storage, demonstrating the potential of hydrosol extract as natural antifungal for strawberry fruits susceptible to decay caused by *B. cinerea.*

Sebaa & *al.* (2019). Have studied the chemical characterization of the essential oils and the hydrosol extract and regional specificity of the major components of *Ballota nigra* essential oil and to evaluate their in vitro and in vivo antifungal activities tested to three phytopathogenic stains (*Penicillium expansum*, *Aspergillus niger* and *Alternaria alternata*). Results: Altogether, 38 compounds were identified in the essential oils. The statistical methods deployed confirmed that there is a relation between the essential oil compositions and the harvest locations. Hydrosol extract was constituted by seven components. The results of in vitro antifungal activity with essential oil and hydrosol extract have shown very interesting antifungal activities on *Penicillium expansum* and *Alternaria alternata* strains with percentage reductions up to 80%. Additionally, in in vivo assays, *Ballota nigra* essential oil and hydrosol extract significantly reduce decay in artificially inoculated tomato by *Alternaria alternata*.

The authors have concluded that the essential oil and hydrosol extract can be used as a potential source of sustainable eco-friendly botanical fungicides to protect stored tomatoes from pathogens, saprophytic fungi causing bio-deterioration to a variety of food commodities.

Boyraz & Özcan (2006), have tested the antifungal activities of the essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) on mycelial growth of *Alternaria mali* Roberts and *Botrytis cinerea* Pers. The results obtained showed that, all doses of extract inhibited 100% the mycelial growth of both fungi, and exhibited a fungicidal effect. 15% level of hydrosol and 1.0% level of ground material had a 100 % effect on *B. cinerea*. Other doses showed weak inhibition on mycelial growth of the fungi, and antifungal activity of the essential oil varied depending on concentrations. While the levels of essential oil show fungistatic effect, the increasing doses of hydrosol and ground material showed a fungicidal effect against *B. cinerea* and *A. mali*. While the ground material had not showed any fungicidal activity against mycelial growth of *A. mali*, the 1% and 1.5% levels of the ground

material exhibited a fungicidal effect on *B. cinerea*. The authors have concluded that, results obtained from this study may contribute to the development of environmentally safer alternatives to protect the spoilage of food products from pathogenic and saprophytic fungi.

Variance analysis has shown very high significant differences between tested volumes for *Botrytis cinerea* versus *L. stoechas* Hydrosol (**Tab. 25**), and no significant defferences for *Botrytis cinerea* versus *P. lentiscus* and *R. graveolens* Hydrosol (**Tab. 26 and 27**).

Table 25: Results of variance analysis of *Botrytis cinerea* versus *L. stoechas* Hydrosol

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	3	224,410	74,8033	89764,00	0 ,000***
Error	8	0,007	0,0008		
Total	11	224,417			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference .

Table 26: Results of variance analysis of *Botrytis cinerea* versus *P. lentiscus* Hydrosol

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	3	225,202	75,0675		NS
Error	8	0,000	0,0000		
Total	11	225,202			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
NS : p>0,05 : No significant difference

Table 27: Results of variance analysis of *Botrytis cinerea* versus *R. graveolens* Hydrosol

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	3	5,06250	1,68750		NS
Error	8	0,00000	0,00000		
Total	11	5,06250			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
NS : p>0,05 : No significant difference

Dunnett tests have shown very high significant differences between 50 µl and 100 µl volumes and control for *Botrytis cinerea* versus *L. stoechas* and *P. lentiscus* hydrosols, and *R. graveolens* at 100 µl, but no significant differences were noticed between control and 25 µl and 100 µl of *R. graveolens* volumes of hydrosols (**Tab.28**).

Table 28: Results of Dunnett tests of *Botrytis cinerea* versus the three hydrosols

Volume of HD / Plants species	**0µl**	**25µl**	**50µl**	**100µl**
Lavandula stoechas **(Means ± σ)**	0 ± 0	0,73 ± 0,057 ***	9 ± 0 ***	9 ± 0 ***
Pistacia lentiscus **(Means ± σ)**	0 ± 0	0,7 ± 1,359 ***	9 ± 0 ***	9 ± 0 ***
Ruta graveolens **(Means ± σ)**	0 ± 0	0 ± 0, NS	0 ± 0, NS	1,433 ± 0,115 ***

NS : No significant (α=0,05)
*** : Very highly significant (α=0,001)

2.3.2.3. Effect of hydrosols on the growth of *Zymoseptoria tritici*

Zymoseptoria tritici was more sensitive to the hydrosols, and it's growth was inhibited by hydrosols of the three plant species studied, notably at volumes of 100 µl of hydrosols **(Fig. 16).** Hydrosol of *P. lentiscus* have recorded the highest value of the diameter of inhibition zone (1.13 ± 0.057 cm), and the most important antifungal activity against *Zymoseptoria tritici* among the three plants. *L. stoechas* and *R. graveolens* displayed slightly lower antifungal activity.

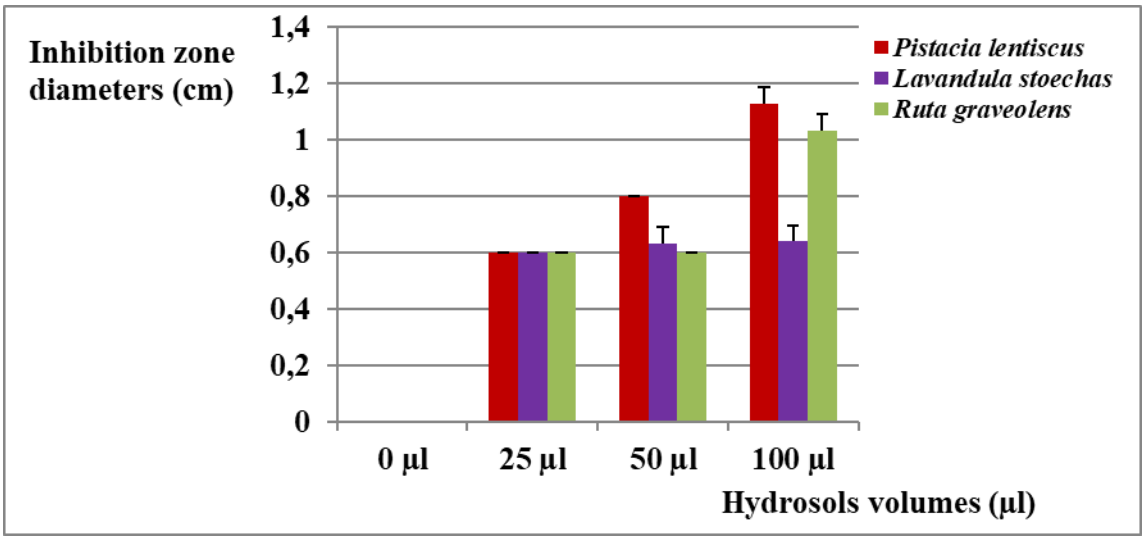

Figure 16 : Diameters of growth inhibition zones of *Zymoseptoria tritici* versus the three hydrosols

According to **D'Amato & *al.* (2018)**, hydrosols are secondary products of hydro-distillation of plants. They exert antibacterial and antifungal activity *in vitro* and *in situ.* Different mechanisms of action related to their chemical composition are proposed. They could be applied in agro-food industry to improve safety and shelf-life. Hydrosols deserve deeper studies to assess application on foods and surfaces. Moreover, their use could be exploited to counteract the phenomenon of antibiotic resistance.

Variance analysis has shown very high significant differences between tested volumes for *Zymoseptoria tritici* versus *L. stoechas* and *P. lentiscus* Hydrosol (**Tab. 29 and 30**), and no significant defferences for *Zymoseptoria tritici* versus and *R. graveolens* Hydrosol (**Tab. 31**).

Table 29: Results of variance analysis of *Zymoseptoria tritici* versus *L. stoechas* Hydrosol

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	3	1,21667	0,405556	243,33	0,000***
Error	8	0,01333	0,001667		
Total	11	1,23000			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference.
*** p<0,001 : Very highly significant difference.

Table 30: Results of variance analysis of *Zymoseptoria tritici* versus *P. lentiscus* Hydrosol

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	3	1,19562	0,398542	273,29	0,000***
Error	8	0,01167	0,001458		
Total	11	1,20729			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference .

Table 31: Results of variance analysis of *Zymoseptoria tritici* versus *R. graveolens* Hydrosol

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	1,62000	0,540000		NS
Error	8	0,0000	0,0000		
Total	11	1,62000			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
NS :Nosignificant difference

Dunnett tests have shown very high significant differences between all volumes and control for *Zymoseptoria tritici* versus all studied hydrosols (**Tab.32**).

Table 32: Results of Dunnett tests of *Zymoseptoria tritici* versus the three hydrosols

Volume of HD / Plants species	0µl	25µl	50µl	100µl
Lavandula stoechas **(Means ± σ)**	0 ± 0	0,6 ± 0 ***	0,633 ± 0,057 ***	1,283 ± 0 ,057 ***
Pistacia lentiscus **(Means ± σ)**	0 ± 0	0,6 ±0 ***	0,8 ± 1,395 ***	1,283 ±0,057 ***
Ruta graveolens **(Means ± σ)**	0 ± 0	0,6± 0 ***	0,6 ± 0 ***	1,0 33 ± 0,057 ***

*** : Very highly significant differences (α=0,001)

2.3.2.4. Effect of hydrosols on the growth of *Fusarium graminearum*

Comparing the average inhibition zone values for *Fusarium graminearum* **(Fig. 17),** we can see, at 100 µL that hydrosols of *L. stoechas* has the highest average inhibition zone with a value of 5 ± 0.000 cm. *P. lentiscus* has the second-highest average inhibition zone with 3 ± 0.000 cm, while *R. graveolens* has the lowest average inhibition zone with 1.533 ± 0.0577 cm.

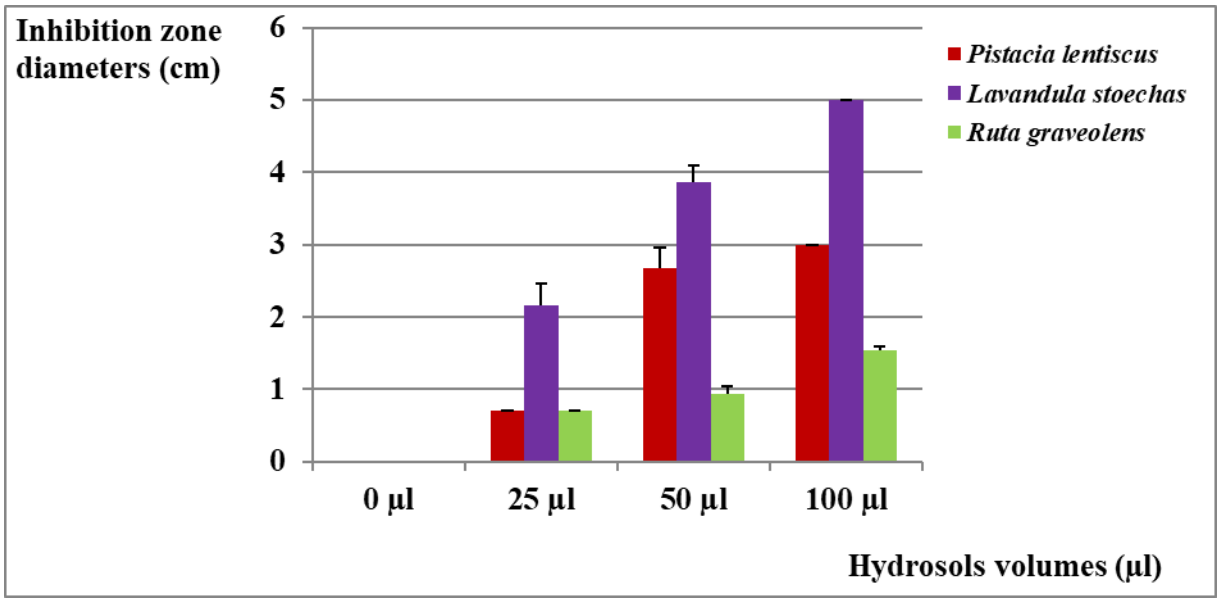

Figure 17 : Diameters of growth inhibition zones of *Fusarium graminearum* versus the three hydrosols

Paramalingam & *al.* (2023) have investigated the antifungal activities of tea tree (*Melaleuca alternifolia*) essential oil (TTO) and hydrosol (TTH) against Fusarium wilt of bananas caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) and their bioactive components. They have found that, compared to the chemical fungicide, TTO effectively suppressed the mycelial growth of *Foc* TR4 at 69%. Both the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of TTO and TTH were established at 0.2 µg/µL and 50 % *v/v*, respectively, suggesting the fungicidal nature of the plant extracts, and results obtained, indicate the potential of tea tree extracts as natural alternatives to chemical fungicides to control *Foc* TR4.

Variance analysis has shown very high significant differences between tested volumes for *Fusarium graminearum* versus *L. stoechas* and *P. lentiscus* Hydrosol (**Tab. 33 and 34**), and no significant defferences for *Fusarium graminearum* versus and *R. graveolens* Hydrosol (**Tab. 35**).

Table 33: Results of variance analysis of *Fusarium graminearum* versus *L. stoechas* Hydrosol

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	3	42,6358	14,2119	14,2119	0,000***
Error	8	0,2733	0,0342		
Total	11	42,9092			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference .

Table 34: Results of variance analysis of *Fusarium graminearum* versus *P. lentiscus* Hydrosol

Sources	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Volumes	3	19,6200	6,54000	373,71	0,000***
Error	8	0,1400	0,01750		
Total	11	19,7600			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
*** p<0,001 : Very highly significant difference .

Table 35: Results of variance analysis of *Fusarium graminearum* versus *R. graveolens* Hydrosol

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	18,54	6,181	1,51	0,284 NS
Error	8	32,73			
Total	11	51,27			

DF : Degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher'sF .
P : Likelihood of highlighting significant difference .
NS : p>0,05 : No significant difference.

Dunnett tests have shown very high significant differences between 50 µL and 100 µL volumes and control for *Fusarium graminearum* versus *P. lentiscus* and *L. stoechas* hydrosols, and no significant differences between control and all tested volumes for *Fusarium graminearum* versus *R. graveolens* hydrosols (**Tab.36**).

Table 36 : Results of Dunnett tests of *Fusarium graminearum* versus the three hydrosols

Volume of HD / Plants species	**0µl**	**25µl**	**50µl**	**100µl**
Lavandula stoechas **(Means ± σ)**	0 ± 0	2,166± 0,288 NS	3,866 ± 0,230 ***	5 ± 0 ***
Pistacia lentiscus **(Means ± σ)**	0 ± 0	0,7 ± 1,359 NS	2,666± 0,288 ***	3± 0 ***
Ruta graveolens **(Means ± σ)**	0 ± 0	0,7 ± 1,359 NS	0,933± 0,115 NS	1,533± 0,057 NS

NS : No significant (α=0,05)
*** : Very highly significant (α=0,001)

Conclusion

Conclusion

Algeria's geographic location grants it a rich and diverse biodiversity, with numerous plant species recognized as valuable sources of bioactive compounds possessing antimicrobial properties.

The aim of this study was to analyze the physicochemical properties of essential oils and hydrosols extracted from three naturally occurring plants in the Guelma region of Algeria *Pistacia lentiscus*, *Lavandula stoechas* and *Ruta graveolens* and to assess their antifungal effectiveness against four common fungal pathogens: *Aspergillus niger*, *Botrytis cinerea*, *Fusarium graminearum* and *Zymoseptoria tritici*, known to cause significant crop losses. The results obtained are summarized as follows:

– A noticeable variation in essential oil yields was observed among the studied plant species, which can be attributed to various factors such as plant species, the specific plant parts used, and growth conditions. *Lavandula stoechas* produced the highest yield at 0.872 %, followed by *Ruta graveolens* with 0.297%. And *Pistacia lentiscus* produced the lowest yield at 0.073%. Overall, substantial differences in essential oil yields were noted between the species.

– The antifungal activity tests of the essential oils revealed significant variations depending on the plant species and the volumes of EOs tested against the target pathogens. At a concentration of 20 µl for all pathogens, the results were as follows:

- *Ruta graveolens* demonstrated the strongest antifungal activity.
- *Lavandula stoechas* showed the second-highest level of effectiveness.
- *Pistacia lentiscus* exhibited the weakest antifungal effect across all tested fungi.

– The antifungal activity tests of hydrosols also revealed slight variations among the plant species and the volumes tested against the target pathogens. At a concentration of 100 µl, the results were as follows:

- *Lavandula stoechas* displayed the highest antifungal activity against all tested pathogens.
- *Pistacia lentiscus* showed the second-highest antifungal activity against *Botrytis cinerea* and *Zymoseptoria tritici*, but exhibited no activity against *Aspergillus niger*.

- *Ruta graveolens* hydrosol showed the weakest overall antifungal effect, except against *Zymoseptoria tritici*.

In summary, the results clearly show that *Ruta graveolens* consistently exhibited the strongest antifungal activity against the tested fungal pathogens (*Aspergillus niger*, *Botrytis cinerea*, *Fusarium gramineum* and *Zymoseptoria tritici*) in essential oil and *Lavandula stoechas* exhibited the strongest antifungal activity in hydrosol forms. *Pistacia lentiscus* showed moderate to low activity against all tested fungi.

From a broader perspective, further research is needed on the chemical composition and potential applications of the essential oils and extracts from these plants. Additional studies on the antimicrobial properties of these and other Algerian plant species will offer deeper insights into their biopesticide potential. Such research could support the development of natural alternatives to synthetic pesticides, contributing to environmentally friendly crop protection and helping to reduce the risk of pest resistance.

Abstracts

Abstract

This research investigates the physicochemical properties and antifungal efficacy of essential oils and hydrosols obtained from *Lavandula stoechas*, *Pistacia lentiscus*, and *Ruta graveolens*, three aromatic plant species naturally occurring in the Guelma region of Algeria. The extracts were tested against four phytopathogenic fungi: *Aspergillus niger*, *Botrytis cinerea*, *Fusarium graminearum*, and *Zymoseptoria tritici*. Essential oils were extracted through hydrodistillation using a *Clevenger*-type apparatus. Among the studied species, *L. stoechas* produced the highest essential oil yield, followed by *R. graveolens*. Antifungal activity was evaluated using the disc diffusion method for essential oils and the well diffusion technique for hydrosols. The results revealed notable antifungal activity for both types of extracts, with *L. stoechas* and *R. graveolens* showing particularly strong inhibition against the target pathogens. These findings support the potential of these natural products as sustainable alternatives for the biological control of fungal diseases in agriculture, contributing to reduced reliance on synthetic fungicides and improved environmental health.

Keywords: *Lavandula stoechas*, *Pistacia lentiscus*, *Ruta graveolens*, Essential oils, Hydrosols, Antifungal activity, *Plant pathogenic fungi.*

Résumé

Cette étude a porté sur les propriétés physicochimiques et l'efficacité antifongique des huiles essentielles et des hydrolats extraits de *Lavandula stoechas*, *Pistacia lentiscus* et *Ruta graveolens*, trois plantes aromatiques qui poussent naturellement dans la région de Guelma, en Algérie. Les extraits ont été testés contre quatre champignons phytopathogènes : *Aspergillus niger*, *Botrytis cinerea*, *Fusarium graminearum* et *Zymoseptoria tritici*. Les huiles essentielles ont été extraites par hydrodistillation à l'aide d'un appareil de type *Clevenger*. Parmi les espèces étudiées, *L. stoechas* a donné le rendement en huile essentielle le plus élevé, suivie de *R. graveolens*. L'activité antifongique a été évaluée par la méthode de diffusion à travers des disques pour les huiles essentielles et la méthode des puits pour les hydrolats. Les résultats ont révélé une activité antifongique notable pour les deux types d'extraits, *L. stoechas* et *R. graveolens* montrant une inhibition particulièrement marquée des pathogènes ciblés. Ces résultats soutiennent le potentiel de ces produits naturels comme alternatives durables pour le contrôle biologique des maladies fongiques en agriculture, contribuant ainsi à réduire la dépendance aux fongicides synthétiques et à améliorer la santé environnementale.

Mots-clés : *Lavandula stoechas*, *Pistacia lentiscus*, *Ruta graveolens*, huile essentielles, hydrolats, activité antifongique, champignons phytopathogènes.

الملخص

تهدف هذه الدراسة إلى تقييم الخصائص الفيزيائية والكيميائية والفعالية المضادة للفطريات للزيوت العطرية والمياه المستخلصة من *Lavandula stoechas* ، و*Pistacia lentiscus*، و*Ruta graveolens* ثلاث نباتات عطرية تنمو بشكل طبيعي في منطقة قالمة بالجزائر. تم اختبار المستخلصات ضد أربعة فطريات ممرضة للنباتات *Aspergillus niger*، *Botrytis cinerea*، *Fusarium graminearum* و *Zymoseptoria tritici*. تم استخراج الزيوت العطرية عن طريق التقطير بالماء باستخدام جهاز *Clevenger* . من بين الأنواع المدروسة، أعطت *Lavandula stoechas* أعلى مردود من الزيت العطري، تليها *Ruta graveolens* . وتم تقييم النشاط المضاد للفطريات باستخدام طريقة الانتشار على الأقراص للزيوت العطرية، وطريقة الآبار لاختبار المستخلص المائي. كشفت النتائج عن نشاط مضاد للفطريات ملحوظ لكلا نوعي المستخلصات، حيث أظهرت *Lavandula stoechas* و *Ruta graveolens* فعالية قوية بشكل خاص ضد الفطريات المستهدفة. تدعم هذه النتائج إمكانية استخدام هذه المنتجات الطبيعية كبدائل مستدامة في المكافحة الحيوية للأمراض الفطرية في الزراعة، مما يساهم في تقليل الاعتماد على المبيدات الفطرية الكيميائية وتحسين الصحة البيئية.

الكلمات المفتاحية : *Lavandula stoechas* ، *Pistacia lentiscus*، *Ruta graveolens*، الزيت الأساسي، المستخلصات المائية، النشاط المضاد للفطريات، فطريات ممرضة للنباتات.

References

References

– **Alami, Y., Lechkar, A., Elbekkali, O., Lakdioui, T., Ibn Mansour, A., Chaoueche, M., & Ouhessine, M. (2016).** Comparative Study of Chemical Composition and the Biological Effect of Essential Oils for Two Plants; *Lavandula Stoechas* et *Laurus Nobilis*. *Maghrebian Journal of Pure and Applied Science*, 2(1), 17- 24.

– **Allagui, M. B., Moumni, M., & Romanazzi, G. (2024).** Antifungal activity of thirty essential oils to control pathogenic fungi of postharvest decay. *Antibiotics, 13* (1), 28.

– **Al-Zaben, M., Zaban, M., Naghmouchi S., Alsaloom A. N., Al-Sugiran N. and Alrokban A. (2023).** Comparison of Phytochemical Composition, Antibacterial, and Antifungal Activities of Extracts from Three Organs of *Pistacia lentiscus* from Saudi Arabia. *Molecules*, 28(13), 5156.

– **Amara, N., Benrima, A., Anba, C., & Belkhir, H. (2019).** Activité antimicrobienne de l'huile essentielle des fruits du pistachier lentisque *(Pistacia lentiscus L.)***.** *Agrobiologia***, 9** (2), 1669–1676.

– **Amhamdi, H., Satrani, B., Ghanmi, M., Aafi, A., Farah, A., Elyacoubi, L., & Chaouch, A. (2009).** Composition chimique et activité antibactérienne des huiles essentielles de *Pistacia lentiscus* L. du Maroc oriental. *Acta Botanica Gallica*, **156** (2), 275–284.

– **Angioni, A., Barra, A., Coroneo, V., Dessi, S., & Cabras, P. (2006).** Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas L. ssp. stoechas* essential oils from stem/leaves and flowers. *Journal of Agricultural and Food Chemistry*, 54 (12), 4364–4370.

– **Attia, E., Z. Abd El-Bakyb R.M., Desoukeya S.Y., Mohamedc M. A., Bishrd M.M., Kamel M.S (2018).** Chemical composition and antimicrobial activities of essential oils of *Ruta graveolens* plants treated with salicylic acid under drought stress conditions. *Future Journal of Pharmaceutical sciences.* 12 p.

– **Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008).** Biological effects of essential oils – A review. *Food and Chemical Toxicology*, 46 (2), 446–475.

– **Belabbes R., Dib M. EL A., Djabou N., Ilias F., Tabti B., Costa J. & Muselli A. (2017).** Chemical Variability, Antioxidant and Antifungal Activities of Essential Oils and Hydrosol Extract of *Calendula arvensis* L. from Western Algeria. *Chemistry & Biodiversity.* Volume14, Issue 5. May, e1600482.

– **Belekar, P., Desai, S., Pathan, S., & Pawar, A. (2024).** Fungal pathogens in crop agriculture: Emerging challenges and advancements in management. *Micro Environem*, 3 (4), 13–19.

– **Benarba, B., & Meddah, B. (2020).** Medicinal plants of North Africa: Algeria. In Benarba B. (Ed.), 2020. *Medicinal and Aromatic Plants of North Africa*, Springer, 27–50.

– **Benmansour, A., Boussahel, S., Kabouche, A., & Touzani, R. (2016).** Chemical composition and biological activities of essential oils of *Ruta officinalis* from Algeria. *Natural Product Research*, 30 (17), 1950–1954.

– **Bouadjina, N. & Louati N. (2023)**. Potential of some medicinal and aromatic plants grown in Algeria as biopesticides. Master's Dissertation, phytopharmacy and plant protection, *University of Guelma* : 55p.

- Bouajaj S., Romane A., Benyamna A., Amri I., Hanana M., Hamrouni L. & Romdhane M. (2014). Essential oil composition, phytotoxic and antifungal activities of *Ruta chalepensis* L. leaves from High Atlas Mountains (Morocco). *Natural product research*, 28 (21), 1910-1914.

– **Boyraz N. & Özcan M. (2006).** Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey. *International Journal of Food Microbiology*. Volume 107, Issue 3, 1 April : 238-242.

– **Cavanagh, H. M. A., & Wilkinson, J. M. (2005).** Lavender essential oil: a review. *Australian Infection Control*, 10 (1), 35–37.

– **D'Amato S., Serio A., Chaves López C., Paparella A. (2018).** Hydrosols: Biological activity and potential as antimicrobials for food applications. *Food Control*. Volume 86, April. 126-137.

– **Dlih Boudiaf, S., Boudarene, L., Zeraib, A., & Boudiaf, K. (2021).** Chemical composition and antioxidant activity of essential oils from *Pistacia lentiscus* L. aerial parts collected from Collo (Skikda, Algeria). *Journal of Essential Oil Bearing Plants*, 24 (5), 1105–1113.

– **Ekwomadu, T. I., & Mwanza, M. (2023)**. *Fusarium* Fungi Pathogens, Identification, Adverse Effects, Disease Management, and Global Food Security: A Review of the Latest Research. *Agriculture*, 13 (9), 1810.

– **El Abdali, Y., Bourhia, M., Moussa, S., Salhi, N., Mechchate, H., Es-Safi, I., … & Bari, A. (2022).** Phytochemical profiling, antifungal, antibacterial, antioxidant, and anti-inflammatory evaluation of *Lavandula stoechas* essential oil grown in Morocco. *Heliyon*, 8 (1), e08786.

– El-Ramady, H., Hajdú, P., Törős, G., Badgar, K., Llanaj, X., Kiss, A. & Prokisch, J. (2022). Plant Nutrition for Human Health: A Pictorial Review on Plant Bioactive Compounds for Sustainable Agriculture. *Sustainability*, 14 (14), 8329.

– **Enayati S., Davari,.M., Yangjeh, A. & Ebadollahi, A. (2019).** Evaluation of growth inhibition effect of essential oils from two *Lavandula* species (*L. stoechas* and *L. officinalis*) on some plant pathogenic fungi. *4th Iranian Mycological Congress, 26-28 August 2019, Sari Agricultural Sciences and Natural Resources University*, Iran. Page 95.

– **Fagbemi, K. O., Aina, D. A., & Olajuyigbe, O. O. (2021).** Soxhlet extraction versus hydrodistillation using the *Clevenger* apparatus: A comparative study on the extraction of a volatile compound from *Tamarindus indica* seeds. *Scientific World Journal*, Article 5961586.

– **Finger, R., Sok, J., Ahovi, E., Akter, S., Bremmer, J., Dachbrodt-Saaydeh, S., … & Möhring, N. (2024).** Towards sustainable crop protection in agriculture: A framework for research and policy. *Agricultural Systems*, 219, 104037.

– **Fones, H. N., & Gurr, S. J. (2015).** The impact of Septoria tritici blotch disease on wheat: An EU perspective. *Fungal Genetics and Biology*, 79, 3–7.

– **Gai, Y., & Wang, H. (2024).** Plant disease: A growing threat to global food security. *Agronomy*, 14(8), 1615.

– **Gardeli, C., Vassiliki, P., Athanasios, M., & Kibouris, T. (2008).** Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: Evaluation of antioxidant capacity of methanolic extracts. *Food Chemistry*, 107 (3), 1120–1130.

– **Garfinkel, A. R. (2021).** The history of *Botrytis* taxonomy, the rise of phylogenetics, and implications for species recognition. *Phytopathology,* 111(3), 437–454.

– **Gautam, A. K., Sharma, S., Avasthi, S., & Bhadauria, R. (2011).** Diversity, pathogenicity and toxicology of *A. niger*: An important spoilage fungi. *Research Journal of Microbiology*, 6 (3), 270–280.

– **Hajji-Hedfi, L., Krifa, M., Bouaziz, A., Mzid, M., & Hosni, K. (2024).** Chemical composition and antimicrobial activity of essential oils from *Pistacia lentiscus* L. leaves collected from different regions of Tunisia. *South African Journal of Botany*, 165, 211–221.

– **Harčárová, M., Čonková, E., Proškovcová, M., Váczi, P., Marcinčáková, D., & Bujňák, L. (2021).** Comparison of antifungal activity of selected essential oils against *Fusarium graminearum* in vitro. *Annals of Agricultural and Environmental Medicine*, *28* (3), 414–418.

– **Isman, M. B. (2020).** Botanical insecticides and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, 65, 233–249.

– **Jaradat, N. (2016)**. Quantitative estimations for the volatile oil by using hydrodistillation and microwave accelerated distillation methods from *Ruta graveolens* L. and *Ruta chalepensis* L. leaves from Jerusalem area / Palestine. *Moroccan Journal of Chemistry*, 4 (1), 1–6.

– **Kannan, R., & Babu, U. V. (2012).** Identity and pharmacognosy of *Ruta graveolens* Linn. *Ancient Science of Life*, 32 (4), 218–221.

– **Katekar, V. P., Rao, A. B., & Sardeshpande, V. R. (2023).** A hydrodistillation-based essential oils extraction: A quest for the most effective and cleaner technology. *Sustainable Chemistry and Pharmacy*, 36, 101270.

– **Kengar, A. A., & Paratkar, G. T. (2014).** Antifungal activity of methanolic extract and essential oil from leaves of *Ruta graveolens* L. against *Candida albicans***,** *Cryptococcus neoformans***,** and *Aspergillus niger***.** *Journal of Ethnopharmacology***,** 151**,** 1–8**.**

– **Khalili, E., Sadravi, M., Naeimi, S., & Khosravi, V. (2012).** Evaluation of the antifungal activity of some plant extracts against the rice blast fungus, *Magnaporthe oryzae*. *Archives of Phytopathology and Plant Protection*, 45 (10), 1167–1175.

- Lian Q., Zhang J., Gan L., Ma Q., Zong Z. & Wang Y. (2017). The Biocontrol Efficacy of *Streptomyces pratensis* LMM15 on *Botrytis cinerea* in Tomato. *Hindawi BioMed Research International*. Volume 2017, Article ID 9486794, 11p. https://doi.org/10.1155/2017/9486794.

– **Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Pérez, C., Colella, M. T., Olaizola, C., & Ontiveros, Y. (2004).** Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, 8 (1), 39–45.

– **Maghnia, D., (2023).** Phytochemicals and Antimicrobial Activity of *Lavandula officinalis* Leaves Against Some Pathogenic Microorganisms. *Egyptian Academic Journal of Biological Sciences.* C, *Physiology & Molecular Biology*. ; 7p.
https://doi.org/10.21608/EAJBSC.2022.292934

- Mezni, F., Aouadhi, C., Khouja, M. L., Khaldi, A. & Maaroufi, A. (2015). In vitro antimicrobial activity of Pistacia lentiscus L. edible oil and phenolic extract. *Na.t Prod. Res.* 29 (6), 565-70. doi: 10.1080/14786419.2014.952232.

– **Mizi , A., Boudiaf, N., Ferchichi,L (2021).** Chemical composition and antioxidant potential of *Pistacia lentiscus* L. essential oil from Oran (Algeria). *Natural Volatiles & Essential Oils Journal*, 8 (4), 1–7.

- Necir K. & Amrani A. (2023). Valorization of some medicinal plants from Guelma region (Algeria), application in plant protection. Master's Dissertation, phytopharmacy and plant protection, *University of Guelma*, 75p.

– **Özcan, M. M., Starovic, M., Aleksic, G., Figueredo, G., Al Juhaimi, F., & Chalchat, J. C. (2018).** Chemical composition and antifungal activity of lavender (*Lavandula stoechas*) oil. *Journal of Essential Oil Research*, 30 (6), 408–413.

– **Paramalingam P. ; Baharum N. A. ; Ong Abdullah J. ; Kyu Hong J. ; Baity Saidi N. (2023).** Antifungal Potential of *Melaleuca alternifolia* against Fungal Pathogen *Fusarium oxysporum* f. sp. *cubense* . Tropical Race 4. *Molecules* 28 (11), 4456.

– **Park, C.H., Park, Y.E., Yeo, H.J., Chun, S.W., Baskar, T.B., Lim, S.S., & Park, S.U. (2019).** Chemical Compositions of the Volatile Oils and Antibacterial Screening of Solvent Extract from Downy Lavender. *Foods*, 132, 1–11.

– **Perczak A. ; Gwiazdowska D. ; Marchwińska K. ; Juś K., Gwiazdowski R. Waśkiewicz A. (2019).** Antifungal activity of selected essential oils against *Fusarium culmorum* and *F. graminearum* and their secondary metabolites in wheat seeds. *Archives of Microbiology* 201, 1085–1097.

– **Pereira, F. O., Mendes, J. M., & Lima, E. O. (2021).** *Ruta* Essential Oils: Composition and Bioactivities. *Molecules*, 26 (16), 4823.

– **Perelló, A., Noll, U., & Slusarenko, A. (2013).** In vitro efficacy of garlic extract to control fungal pathogens of wheat. *Journal of Medicinal Plants Research*, 7 (24), 1819–181

– **Petrikkou, E., Rodríguez-Tudela, J., Cuenca-Estrella, M., Gómez, A., Molleja, A., & Mellado, E. (2001).** Inoculum Standardization for Antifungal Susceptibility Testing of Filamentous Fungi Pathogenic for Humans. *Journal of Clinical Microbiology*, 39 (4), 1345–1347.

– **Remmal, A., Bouchikhi, T., Rhayour, K., Ettayebi, M., & Tantaoui, A. (2014).** Improved Method of the determination of antimicrobial activity of essential oils in Agar medium. *Journal of Essential Oil Research*, 5 (2), 179–184.

– **Sebaa N. ; Zatla A.T. , Dib M. EL A., Tabti B., Costa J. & Muselli A. (2019).** Antifungal Activity of Essential Oil and Hydrosol Extract of *Ballota nigra* L. and their Protective Effects Against the Black Rot of Tomatoes. *Current Nutrition & Food Science*. Volume 15, Issue 7, 662 – 671.

– **Soylu, E. M., Soylu, S., & Kurt, S. (2010).** In vitro and in vivo antifungal activities of the essential oils of various plants against tomato gray mold disease agent *Botrytis cinerea*. *International Journal of Food Microbiology*, 143 (3), 183–189.

– **Stępień, Ł. (2020).** *Fusarium*: Mycotoxins, Taxonomy and Pathogenicity. *Microorganisms*, 8 (9), 1404.

– **Van Kan, J. A. L. (2006)**. Licensed to kill: The lifestyle of a necrotrophic plant pathogen. *Trends in Plant Science*, 11 (5), 247–253.

– **Williams, A., Sinanaj, B., & Hoysted, G. A. (2024).** Plant–microbe interactions through a lens: Tales from the mycorrhizosphere. *Annals of Botany*, 133 (3), 399–412.

– **Zheljazkov V.D. & Craker L.E. (2016).** Overview of medicinal and aromatic crops. Chapter 1 *in* Zheljazkov and Cantrell (2016). Medicinal and Aromatic Crops: Production, Phytochemistry, and Utilization. *ACS Symposium Series; American Chemical Society*: Washington, DC, 12p.

– **Zuzarte, M., Gonçalves, M. J., Cavaleiro, C., Cruz, M. T., Pinto, E., Vale-Silva, L., Salgueiro, L. (2013).** Chemical composition and antifungal activity of the essential oils of *Lavandula viridis* L'Hér. *Journal of Medical Microbiology***,** 62 (5), 876–884.

Web sites :

[1] : https://www.britannica.com/science/fusarium-wilt (view on 30/04/2025).

[2] :https://www.discoverthegreentech.com/fongicide-ogm-fusariose/ (view on 20/05/2025).

[3] :https://www.ipmimages.org/browse/detail.cfm?imgnum=5367344 (view on 30/05/2025).

[4] :https://www.gardenersworld.com/how-to/solve-problems/grey-mould-on-soft-fruits/ (view on 25/05/2025).

[5] : http://ephytia.inra.fr/fr/C/7309/Aubergine-Botrytis-cinerea (view on 10/05/2025).

[6] : https://www.cropsmart.com.au/keep-an-eye-out-for-septoria-tritici-blotch/ (view on 10/05/2025).

[7] : https://www.wildflowersprovence.fr/media/plants/pistacia-lentiscus-2edcu-900x675.jpg (view on 30/04/2025).

[8] :https://cdn.hillier.co.uk/wpcontent/uploads/2021/05/lavandula%20stoechas%20papillon.jpg (view on 20/03/2025).

[9] : https://budgetseeds.co.uk/wp-content/uploads/2023/11/rue.jpg (view on 30/03/2025).

[10] :https://weatherspark.com/y/55170/Average-Weather-in-Guelma-Algeria-Year-Round (view on 02/05/2025).