الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي و البحث العلمي

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

UNIVERSITY MAY 8, 1945 GUELMA
FACULTY OF NATURAL AND LIFE SCIENCES AND EARTH AND UNIVERSE SCIENCES

Master's Dissertation

Domain: Natural and Life Sciences

Sector: Agricultural Sciences

Specialty/ Option: Phytopharmacy and plant protection

Department: Ecology and Environmental Engineering

Valorization of some medicinal plants from Guelma region (Algeria), application in plant protection

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June 2023

Acknowledgments

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

We begin this dedication by acknowledging the grace and blessings of Allah, the Almighty, whose guidance and mercy have illuminated our path throughout this journey. It is with deep gratitude and reverence that we attribute every step of our success to His divine guidance.

We welcome the opportunity of achieving this work to acknowledge a number of individuals:

First, we extend our deepest gratitude to our esteemed Supervisor, **Dr. ALLOUI Nora**, whose guidance, mentorship, and unwavering belief in our capabilities have been instrumental in shaping our academic and intellectual growth. Your wisdom, expertise, and constructive feedback have challenged us to surpass our own limitations and strive for excellence. Your dedication to nurturing talent and fostering a love for learning has left an indelible mark on our journey, and we are honoured to have been under your tutelage.

Our sincere acknowledgements go to **Dr. OUCHTATI Nadia** and **Dr. CHAHAT Nora** who have been so kind to accept to be members of the jury and to read our work.

We also would like to express our sincere thanks to **Dr. KSOURI Samir** and Professor **BENHAMIDA Aida** for their help in practical parts of the work, and **Dr. ZITOUNI Ali**, for his help for statistical treatments of the results.

We are also deeply grateful to Miss **LOUIZA**, responsible of laboratory 08 for her help and her moral support.

We would also thank all our family members for their efforts to provide us with the most suitable conditions for success. Their encouragements have always been a good drive to us to accomplish this work. Thank you all.

Our thanks go also to the vast Scientific Community, whose pursuit of knowledge and passion for discovery has inspired us, we dedicate this work to you. It is through our collective efforts and contributions as researchers, scholars, and scientists that progress and innovation thrive. May this work serve as a humble contribution to the ever-growing body of knowledge, fostering dialogue, and pushing the boundaries of understanding.

And lastly, we express our heartfelt appreciation to the institution that has been the foundation of our educational endeavors, the University of 08 May 1945 Guelma. Your commitment to academic excellence, interdisciplinary learning, and personal growth has enriched our university years in profound ways. The nurturing environment, diverse perspectives, and countless opportunities for growth have shaped us into the individuals we are today. We are forever grateful for the knowledge, experiences, and friendships forged during our time here.

Dedications

I humbly dedicate this work to my beloved family, friends, and esteemed supervisor :

- To my beloved family, In the name of Allah, the Most Gracious, the Most Merciful, I dedicate this heartfelt tribute to each of you for your unwavering love, support, and belief in my journey.
- To my dear mother, Ouahiba, who embarked on the sacred journey of Hajj, your love, guidance, and sacrifices have shaped the person I am today. You have given me the strength to overcome challenges and reach for the stars. Your presence is a beacon of light that illuminates my path.
- To my late father, Saddek, who may no longer be with us physically, but whose spirit and teachings continue to guide me, I honor your memory. Your wisdom, encouragement, and unconditional love have left an indelible mark on my heart. I carry your values and life lessons with me, cherishing the time we shared and the values you instilled in me.
- To my twin sister, Amna, my soulmate and confidante, this dedication is a celebration of our unique connection. Your support, understanding, and love have been a constant source of comfort and motivation. Our journey together is filled with shared laughter, tears, and unforgettable moments that I will cherish forever.
- To my brother Ahmed Hacen and my little sister Abir, To my uncle, Hicham, my grandparents, and the rest of my family, whose love, wisdom, and guidance have shaped my values and provided a strong foundation
- To my dear friends, Amani and Nadra, who have been pillars of strength and companions on this journey, I dedicate this work to you.
- To my partner and best friend, Amsa, who has been my rock and confidante, your love, encouragement, and belief in my abilities have been instrumental in my journey.
- Lastly, to my esteemed supervisor, Dr. Allioui Nora, who has guided me with her wisdom, expertise, and unwavering support, I extend my deepest gratitude. Your mentorship, encouragement, and belief in my potential have pushed me to surpass my own limitations and strive for excellence. Your dedication to my growth and success is deeply appreciated.
- To each and every one of you, I am grateful for your presence in my life. Your love, support, and belief in me have shaped my journey and made me the person I am today. May our bond continue to strengthen, and may we celebrate many more milestones together.
- I dedicate this work to you with heartfelt appreciation and love.

NECIR Khaoula

Dedications

I humbly dedicate this work:

- To my family, who gave me a dignified education, her love has made me what I am today, especially:

- To you my dearest father **NACER ,** to the man of my life, my eternal example, my moral support, my source of joy and happiness, the one who has always sacrificed himself to see me succeed, may God grant you a long life so that you may witness my success, proud of you daddy.
- To my dear dear mother **HANANE** ,the woman who suffered without letting me suffer, in the light of my days, the source of my efforts, the flame of my heart, my life and my happiness; who compensated me for all the tenderness of the world and embraced my dreams and grief, my soulmate, sister, secretive partner and everything beautiful in my life. All words are unable to express how much I love you mommy.
- My brother and my darling **Amir** you are something special in my life you are the beautiful compensator, you are the source of my joy every day, I am grateful to God for having a brother like you, I wish you success and success in the baccalaureate certificate. I love you Mirou .
- To my little siblings **Asomi** and **Walid**, you are the joy of our house and the playful spirit... You wouldn't have completed the house without you, and the mess you make is the pleasure of the house. Thanks for your positive energy you give me.
- To my grandmother, uncles and aunts, thank you for your love and encouragement.

- To my friend **Khawla** and dearest friend to my heart, thank you for being with me and standing by me in all circumstances. I am fortunate to have a friend like you. Thank you for sharing this work with me.

- To our supervisor **Dr. ALLIOUI Nora**, I warmly thank her for her kindness, her confidence, her rigour, her valuable advice and for the time she has given us despite her obligations and responsibilities, during the preparation of this brief.

- To all my friends and comrades of the class 2022/2023: For their company and the good times spent together during the last 6 years, I wish you good luck and more success.

AMRANI Asma

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General Introduction

General introduction

Plants make up the majority of the earth's living environment as trees, grass, flowers, and so on. Directly or indirectly, plants also make up all the food on which humans and all animals depend. Even the meat, milk, and eggs that we and other carnivores eat come from animals that themselves depend on plants for their food. However, Plant diseases, by their presence, prevent the cultivation and growth of food plants in some areas; or food plants may be cultivated and grown but plant diseases may attack them, destroy parts or all of the plants, and reduce much of their produce. It is conservatively estimated that diseases, insects, and weeds together annually interfere with the production of, or destroy, between 31 and 42% of all crops produced worldwide (**Agrios , 2005**).

Crop failures were common in ancient times and throughout the Middle Ages, plant diseases were frequently blamed on the displeasure of various deities (**Windham & Windham, 2008**). Over $220 billion is lost annually to plant diseases [1].

Fungi have the ability to destroy crops, and the economic consequences have been enormous throughout human history. They reduce crop yield, destroy crops in the field and during storage, and produce toxins that are toxic to humans and animals (**Gould, 2017**). Fungal pathogens account for 70-80% of agricultural production losses caused by microbial diseases. There are approximately 8,000 fungal species that cause 100,000 plant diseases (**Bhandari & *al.*, 2021**).

Many plants pathogenic fungi, causes common diseases, and among them certain members of *Aspergillus* genus, *Botrytis cinerea* and *Zymoseptoria tritici,* are responsible of serious damages of crops anywhere in the world.

Aspergillus species are geographically widely distributed and have been observed in a broad range of habitats because they can colonize a wide variety of substrates (**Sharma, 2012**). They are well-known agricultural pests and, more importantly, producers of various mycotoxins that endanger food safety worldwide. *Aspergillis* can switch between saprophytic and pathogenic lifestyles, and secondary metabolites production, such as mycotoxins, can vary according to these fungal lifestyles (**Pfliegler & *al.*, 2020**).

Aspergillus niger is an ascomycota fungus, belonging to the subphylum Pezizomycotina, class Eurotiomycetes, order Eurotiales, and family of Aspergillaceae (**Schoch & *al.*, 2020**). It is one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions and peanuts and is a common contaminant of food. It is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles and other decaying vegetation (**Sharma, 2012**). For many plants, *Aspergillus niger* is a dangerous pathogen. It is a major cause of plant tissues destruction, rotting, and decomposition (**Tawfik & *al.*, 2022**), particularly if temperatures are extremely high when the crops are mature or in storage. The

disease can spread within the crop as well as to neighbouring crops via airborne spores carried by the wind during harvesting. Although *A. niger* is known to be seed-borne, soil is also an important source of inoculation. On onion, the presence of powdery mould spores on the surface or between the scales of the bulb is the most notable feature (**Fig. 01**). Soft rot can appear in varying degrees on affected bulbs (**Syngenta, 2020**).

Figure 01: Black mold caused by *Aspergillus niger* on onion **[2]**

Gray mold rot, also called gray mold blight or botrytis blight, is another disease of plants growing in humid areas caused by fungi in the genus *Botrytis*, usually *B. cinerea* (**Britannica, 2019**).

Botrytis is a highly ranked plant pathogen due to its broad host range that includes hundreds of plants. Such hosts are in almost all commodity groups: annuals and perennials, herbaceous and woody plants, food and ornamental crops, vegetable and fruit and field crops. Included within this diverse list are dozens of high value vegetable, fruit, and ornamental commodities (bean, Citrus, grape, cherry, lettuce, onion,…), for which gray mold can inflict sizeable economic losses [3].

Gray mold is a fungal disease that travels quickly through gardens, especially during damp, cool to mild weather. Symptoms appear as grayish-colored soft, mushy spots on leaves, stems, flowers and produce (**Figs. 02 and 03**). Spores develop when conditions are optimal and are moved by wind or splashing water onto blossoms or young leaves, where they germinate and enter the

plant. Germinating spores rarely penetrate green, healthy tissue directly, but can enter through wounds on growing plants. Cuttings are particularly susceptible to infection (**Vinje, 2019**).

Figure 02: Strawberry fruit infected by gray mold [**3**].

Figure 03: Gray mold on grapes [**4**].

Botrytis cinerea (teleomorph: *Botryotinia fuckeliana*) is also an Ascomycota and Pezizomycotina fungus, of Leotiomycetes class, order of Helotiales, family of Sclerotiniaceae. It is a necrotrophic airborne plant pathogen that attacks over 200 crop hosts worldwide. Although fungicides are available to control it, many classes of fungicides have failed due to its genetic plasticity (**Williamson & *al.*, 2007**).

Zymoseptoria tritici (teleomorph *Mycosphaerella graminicola*), is the causal agent of one of the most devastating foliar diseases of wheat: Septoria tritici Blotch (**Fig. 04**). It is a notable pathogen of wheat grown in temperate climates throughout the world (**Fones & Gurr, 2015**). This fungus belongs to the Ascomycota phylum, Subphylum of Pezizomycotina, Class of Dothideomycetes, Subclass of Dothideomycetidae, Order of Mycosphaerellales, Family of Mycosphaerellaceae and Genus of *Zymoseptoria* [**5**]. Losses due to this disease can reach up to 50% in epidemic years, and often vary between 5 and 20% depending on the environment and the cultivar of wheat; it has been estimated that up to 70% of fungicide use in Europe is to control this disease (**Fones & Gurr, 2015;Torriani & *al.*, 2015**).

Figure 04: Septoria leaf blotch of wheat (Kuzdraliński, et al., 2020).

Because of the increased demand for food to feed the world's ever-growing population, synthetic chemicals (pesticides) were developed and adopted as a quick and effective strategy for managing crop pests and diseases (**Lengai & *al.*, 2020**).

Pesticide, by definition is any toxic substance used to kill animals, fungi, or plants that cause economic damage to crop or ornamental plants or endanger domestic animal or human health. (**Britannica, 2023**). Overreliance on synthetic pesticides is discouraged due to their detrimental effects on human health, the environment, and development of resistant pest and pathogen strains (**Lengai & *al.*, 2020**).

Controlling plant diseases often necessitates the application of such toxic chemicals not only on plants and plant products that we consume, but also into the soil, where many pathogenic microorganisms live and attack the plant roots. Many of these chemicals have been shown to be toxic to nontarget microorganisms and animals and may be toxic to humans (**Agrios, 2005**).

Ahmed & *al.* (2023) reports that synthetic fungicides have long been used to effectively control fungal diseases. However, their extensive use and residual effects in soil, plant, and water have led to many serious side effects, including environmental disorders, human health risks, damage to aquatic ecosystems, reduction of beneficial soil microorganisms, development of fungicide-resistant fungi, and depletion of ozone layer.

The short- and long-term costs of environmental contamination on human health and welfare caused by our efforts to control plant diseases (and other pests) are difficult to estimate. Much of modern research in plant pathology aims at finding other environmentally friendly means of controlling plant diseases (**Agrios, 2005**), and there is an urgent need to develop new safe and effective alternatives for the management of fungal diseases. Among these alternatives are plant extracts, essential oils, and isolated bioactive compounds. Medicinal and aromatic plants are renewable natural sources full of bioactive substances belonging to different chemical classes. Previous studies of antimicrobial properties show the potential of plant extracts and essential oils of these plants as antimicrobial, antibacterial, antifungal, and antiviral **(Ahmed & *al.*, 2023)**.

Botanical fungicides may be a viable and sustainable option in this regard. Several studies have shown that phyto-chemicals have fungicidal properties. They are easily degradable, retain soil properties, and are both environmentally and human-safe (**Bhandari & *al.*, 2021)**. Botanical pesticides are effective in controlling various crop pests, inexpensive, easily biodegraded, have multiple modes of action, are readily available, and have low toxicity to non-target organisms (**Lengai & *al.*, 2020**).

The United States Environmental Protection Agency (US EPA) classifies biopesticides as microbial pesticides, plant-incorporated protectants, and biochemical pesticides. Growth regulators, pheromones, oils, soaps, and minerals are examples of biochemical (**Agrow, 2012**).

Essential oil (EO) is a highly volatile substance extracted by physical means from a single botanical species odoriferous plant. Distillation is the most common method for isolating essential oils, but other methods, such as effleurage (fat extraction), maceration, solvent extraction, and mechanical pressing, are used for specific products. Younger plants produce more oil than older plants, but older plants have more resinous and darker oils because the lighter fractions of the oil continue to evaporate. Only a few thousand-plant species have essential oils that have been thoroughly characterized and identified. Oils are stored as microdroplets in plant glands. After diffusing through the gland walls, the droplets spread across the plant's surface before evaporating and filling the air with perfume (**Britannica, 2023**).

According to **Martinez (2012**), these oils are commonly found in the composition of plants of the Lamiaceae family (e.g., mints *Mentha* sp., and oregano *Origanum* sp.,). The essential oils of many plants have antimicrobial properties (**Alekseeva & *al*., 2020; Berka-Zougali & *al*., 2012; Chouhan & *al.*, 2017; Hammer & *al*., 1999**).

The essential oils' fungicide and fungistatic activities, as well as the growing literature on their mechanisms of action and knowledge about their traditional and new uses, highlight the potential applications of these natural substances in many fields, ranging from human medicine to agriculture, food technology, and the reduction of the use of synthetic drugs and additives (**Nazzaro & *al*., 2017).**

Terpenes make up a large portion of the essential oils isolated from mints (**Martínez, 2012**) and the antimicrobial or antifungal activity of essential oil may be due to the properties of terpenes/terpenoids, which are capable of disrupting the cell membrane, causing cell death, or inhibiting the sporulation and germination of food spoilage fungi due to their highly lipophilic nature and low molecular weight (**Nazzaro & *al*., 2017).**

In Algeria, collection of medicinal and aromatic plants to extract, after distillation, essential oils for the manufacture of cosmetics, pharmaceuticals as well as flavors for food products, is a virgin field. The distillation of plants is sufficiently known, but remains largely untapped, despite the availability in Algeria of large tracts of forests and fields, whose territory covers important plant resources distributed on the coasts, plains, mountains, steppes, the Sahara and around water points **(Reguieg, 2011).**

In this research, we will be using three common plants in Algeria: Mints, oregano and myrtle.

Mints are perennial, rarely annual, aromatic plants which belong to more than 18 species and 11 hybrids in the genus *Mentha* that is distributed across Africa, Asia and North America. The genus *Mentha* is a member of family Lamiaceae, order Lamiales (**Salama & *al.*, 2019**). They are wide-spreading underground and over ground stolon's and erects, square, branched stems. Eessential oils of some *Mentha* species from different geographical regions have shown insecticidal (anti-feedant, repellent, and ovicidal) and antimicrobial efficacies against bacterial, fungal plant pathogens and insects **(Kayser, 2021).**

Mentha rotundifolia (L.) Huds. (**Fig. 05**), member of the Lamiaceae family, is a perennial herbaceous variety that grows wild and has strong aromatic properties. This endemic shrub is well known in Algeria and North Africa under the name "timarssaad." It is well liked and valued in traditional Algerian therapy **(Brada *&* *al.*, 2006).**

Figure 05: *Mentha rotundifolia* leaves [**6**]

The *Myrtaceae* is an ecologically important angiosperm family containing both trees and shrubs and has its name taken from the shrub 'Myrtus' which is found near the Mediterranean in North Africa and in South America. The *Myrtaceae* is a dicot family in the class Rosidae (**Mitra & *al.*, 2012).**

Myrtle (*Myrtus communis* L.) is a typical plant of the coasts of Mediterranean area, such as North Africa or Southern Europe and also West Asia. Common Myrtle belongs to the Myrtaceae

family with some 145 genus and over 5500 species **(Snow & *al*., 2011),** and grows spontaneously as an evergreen shrub or a small tree. The plant can reach a height of 2.5 m, with a full head deeply covered by branches and small leaves; flowers are starry, scented, and can be white or pink. Whereas berry fruits are edible, small, with a round shape and many seeds inside, generally blue–black (**Fig. 06**), even if some varieties have white–yellow fruits, and ripen in autumn, between October and February (**Petretto & *al*., 2016**).

Figure 06: Myrtle plant *Myrtus communis* [**7**]

In Algeria, the myrtle plant (*Myrtus communis* L.), known as "Al-Rihan" or "el-halmouche" grows very well in many areas, on mounds or hills, in coastal or in more remote areas, and it is currently generating real interest regarding its use as a medicinal plant. Myrtle flowers, leaves and berries are used for external applications to heal wounds, for skin diseases (psoriasis, herpes, bruises etc.) and for internal functions to treat many diseases such as dysentery, urinary tract infections, hemorrhoids, and even hair loss. In some areas, its use is recommended to lower blood sugar as well as to improve digestion (**Berka-Zougali & *al*., 2012**). *M. communis* EO has been used as an anti-inflammatory, antimutagenic, anti-diabetic, anti-malarial, and anti-protozoal agent. Furthermore, it has shown an insecticidal effect and antioxidant activity. The herb is used traditionally for the treatment of disorders such as diarrhea, peptic ulcer, hemorrhoid, inflammation, pulmonary and skin diseases, although clinical and experimental studies suggest that it possesses a broader spectrum of pharmacological and therapeutic effects such as antioxidative, anticancer, antiviral, antibacterial, and antifungal activity (**Alipour & *al*., 2014**). **Zine Laabidine & *al*., (2021)**

suggests that the antifungal activity of *Myrtus communis* L. essential oil could have potential applications in the development of biofungicides and as a preservative in the food industry.

Oregano (*Origanum* sp.) is a flowering plant genus with over 50 species that belongs to the Lamiaceae family (**Alekseeva & *al.*, 2020**). Oregano is native to the hills of the Mediterranean countries and western Asia and has naturalized in parts of Mexico and the United States. Oregano is usually grown as a small evergreen subshrub in mild climates. Its compact oval leaves (**Fig. 07**) are arranged oppositely and are covered with glandular trichomes (plant hairs). The young stems are typically square and hairy and become woody with age. The flowers are small and borne in clusters; they range in colour from white to pink or pale purple (**Britannica, 2023**).

Figure 07: Origano plant *Origanum floribundum* [**8**]

Oregano is used as a culinary and medicinal herb. It's also popular as a decorative and melliferous plant. It blooms from June to the end of the vegetation and produces fruit and seeds at the same time beginning in August. This species is quite adaptable; it produces a variety of morphologically and chemically differentiated forms, which are related to their location of occurrence **(Nurzyńska-Wierdak, 2009).**It has many beneficial properties, including antibacterial, antifungal, antioxidant, anti-inflammatory, antitumor, and antiviral. While a variety of components found naturally in these plants provide a variety of benefits, phenolic compounds in particular are important for biocidal and antioxidant properties (**García-Beltrán & Esteban, 2016**).

Aromatic plants are processed by different distillation techniques, and distillation process generates five distinct products: essential oil, hydrosol, distilled biomass, residual water and plant ash from the furnace **(Rao, 2013).**

During distillation, a small hydrophilic fraction of polar, oxygenated, odor imparting, water-soluble oil constituents escapes into the distillation / condensate water stream. The condensate water with dissolved oil components is known as hydrosol. This fraction is high in organoleptically valuable oxygenated compounds, which enhance the flavor value of essential **oils (Rao, 2012).** Perfumery, cosmetics, food flavoring, aromatherapy, and traditional therapies all make use of hydrosols. Hydrosols have biological activities and have the potential to be global economic commodities. These hydrosols are discarded and thus wasted in many EO producing and exporting countries **(Rao, 2013).**

The present study focuses on the yield of essential oils, and antifungal activity of essential oils and hydrosols of aerial parts from different aromatic plants grown in Guelma region located in the North-East of Algeria (*Origanum floribundum, Mentha rotundifolia* and *Myrtus communis*), against three plant pathogenic fungi (*Zymoseptoria tritici, Botrytis cinerea* and *Aspergillus niger*), in order to investigate more about the possibility of including their essential oils or hydrosols as botanical pesticides, reduce synthetic use and protect the environment, non-target organisms, and human health due to their residual effects.

Material & Methods

Chapter 01: Material and methods

Objectives of the study

The present work means to evaluate the biological activities of many aromatic and medicinal plants collected from Guelma region (North-Est of Algeria) by the determination of:

- Essential oil's (EOs) yield.
- EOs and Hydrosols characteristics
- Antifungal activity of EOs and Hydrosols.

1.1. Plant material

Our research focused on the aerial parts of three aromatic and medicinal plant species:

- ❖ Mint (*Mentha rotundifolia*)
- ❖ Myrtle (*Myrtus communis*)
- ❖ Oregano (*Origanum floribundum Munby*)

Table 01 presents more details on plant material used in this study.

Table 01: Origins and characters of plant material used

Species	Family	Date of collection	Location	Parts used
Mentha rotundifolia	Lamiaceae	June 2022	Guelaât BouSbaâ (Guelma) - Altitude 36°32'44" North - Longitude 7°28 '24" East	Leaves and floral summits
Myrtus communis	Myrtaceae	March 2023	Beni-Salah Forest (Guelma) - Altitude 36°47'43" North - Longitude 7°83 '89" East	Leaves and fruits
Origanum floribundum Munby	Lamiaceae	April 2022	Djebel Houara (Guelma) - Altitude 36°32'36"North - Longitude 7°31' 33"East	Leaves and floral summits

The plant species were chosen for the following reasons:

- ❖ Their widespread use in traditional medicine (preparation of herbal teas and other products), for the treatment of human microbial diseases and infections.
- ❖ Their broad distribution as natural botanical resources throughout the country, particularly in eastern Algeria.

- Lack of researches on biopesticide properties, particularly the antifungal ability of these plant species' essential oils.

1.1.1. Geographical situation of the areas collection of plant material

Guelma, origin of the plant species used, is located in northeast Algeria, at an altitude of 36°27'43" North and a longitude of 7°25'33" East. The elevation is 305 meters above sea level. The Wilaya's geography is distinguished by a varied relief, with an emphasis on an important forest cover and the Seybouse passage, which serves as the main waterway.

Wilaya's territory has a sub-humid climate in the center and north, and a semi-arid climate in the south. In the winter, it is mild and rainy, and in the summer, it is warm. The temperature typically varies from 4°C to 35°C and is rarely below 0°C or above 39°C **[9].**

1.1.2. Samples treatment

The collected plants were cleaned and dried in a well-ventilated area and at room temperature for a period of 07 to 15 days depending on the species, After drying, the samples, isolated from the rest of the plant parts were kept in clean bags until the time of use.

1.1.3. Extraction of essential oils

Essential oils (EOs) were extracted by hydrodistillation using a *Clevenger* type apparatus (**Fig. 08**) at the laboratories of the Faculty of Natural and Life Sciences and Earth and Universe Sciences of 8th May 1945 University of Guelma.

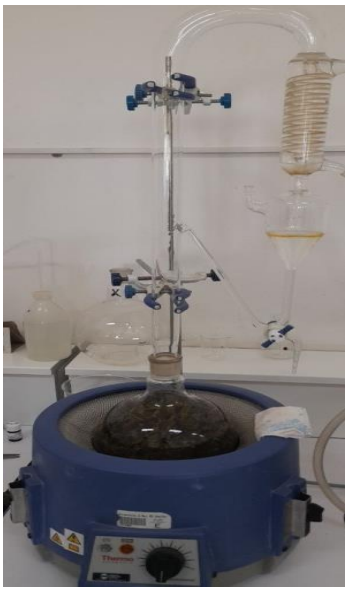

Figure 08: *Clevenger* apparatus used for essential oils extraction

Hydrodistillation, is the most used technique in research laboratories to capture small amounts of volatile compounds from aromatic plants and to determine the essential oil content of plant materials. The distillation apparatus, published by Clevenger in 1928, has later found various modifications. The modified device has been adopted by the European Pharmacopoeia and several other pharmacopoeias. It is made up of a heated round bottom device that contains ground plant material and water and is linked to a vertical condenser and a graduated tube. A three-way valve at the bottom of the tube allows the water to be directed to the flake while separating the essential oil from the aqueous phase **(Kubeczka, 2010**).

1.1.4. Preservation of the essential oils obtained and hydrosols

Essential oils and hydrosols of the different plant species used in this study were collected separately in clean, aseptic containers, and stored in a cool place (congener for the EOs and freezer for hydrosols).

1.1.5. Determination of yield's essential oils

Essential oil yield is defined as the ratio of the mass of essential oil obtained after extraction to the mass of dry vegetable material used. It is expressed as a percentage and calculated using the formula reported by **Samadi & *al.* (2020**).

$$Y_{EO} = \frac{Mass\ of\ EO\ (in\ grams)}{Mass\ of\ dried\ plant\ (in\ grams)} \times 100$$

Y_{EO}: Essential oil yield (*in percentage*).

1.2. Fungal material

The study is focused on three plant pathogenic fungi species:

- *Zymoseptoria tritici*
- *Botrytis cinerea*
- *Aspergillus niger*

1.2.1. Sample collection

Zymoseptoria tritici was isolated from common wheat leaves, shown Septoria tritici blotch symptoms, while *Botrytis cinerea* was isolated from infected fruit of strawberry with gray mold symptoms. *Aspergillus niger* was isolated previously from contaminated vegetables by Dr. Allioui N. and conserved as the laboratory's collection.

1.2.2. Cultivation and preservation of strains

Fungal strains were grown on the nutrient medium PDA (Potato Dextrose Agar), incubated for 05 days (for *Botrytis cinerea* and *Aspergillus niger*) to 07 days (for *Zymoseptoria tritici*) at 24°C, and then stored at + 4°C until use.

1.3. Antifungal activity tests

1.3.1. Material

- Incubator, set at 24 °C.
- Micro pipettes (10, 100 and 1000 µL) + tips
- Physiological water solution
- Optical microscope
- Tween 80
- Vortex
- Culture media: PDA
- Wattman paper discs (6 mm of diameter)
- Petri dishes (90 mm of diameter)
- Samples of EOs and Hydrosols
- Pasteur Pipettes

1.3.2. Preparation of spore suspensions

Sporal suspensions for the various fungal strains were prepared from young cultures, 5 days old for *Botrytis cinerea* and *Aspergillus niger*, and 7 days old for *Zymoseptoria tritici*. The spores were obtained by scraping them from 15 mL sterile plastic tapered tubes containing 0.9% physiological water solution and 2 drops of Tween 80. The concentrations are adjusted to specific values cited in literature reports, for the different 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.,* 2018**).
- For *Aspergillus niger*: a sporal concentration of 10^6 spores/mL (**Petrikkou & *al.,* 2001**).

1.3.3. Fungal Strains-Essential Oils Susceptibility test

For the test of essential oils susceptibility of studied fungal strains, we used four volumes of pure essential oils: 10 µL, 20 µL, and 40 µL, as well as a negative control (0 µL) treated with sterilized distilled water.

The disk diffusion method was used. It's the most commonly used method for determining antimicrobial susceptibility patterns **(Khalili *& al.,* 2012**). It is a direct confrontation technique with diffusion through Wattman paper discs, and it consists of the oils diffusing in the culture medium inoculated by the fungi being studied via 6 mm diameter wattman paper discs placed in the center of the Petri dishes: 500 µL of sporal suspension, prepared the same day, is spread uniformly over the medium in each 90 mm diameter Petri dish containing the PDA culture medium. After drying for few minutes, a 6 mm diameter Wattman paper disc is placed in center and soaked with the desired concentration of the essential oil to be tested. After that, Petri dishes are incubated at 24°C. The results are read by examining and measuring the diameter of the fungal strain's growth inhibition zone in the vicinity of the discs charged by the various volumes of the EOs tested (after 3 days for *Botrytis cinerea* and *Aspergillus niger*, and six days for *Zymoseptoria tritici*).Three replicates were used for all volumes of all treatments.

1.3.4. Fungal Strains-Hydrosol Susceptibility test

For fungal strain-Hydrosol susceptibility test, we used three volumes of pure hydrosol: 25µL, 50 µL, and 100 µL, as well as a negative control (0 µL) treated with sterilized distilled water. Three replicates were used for all volumes. The well diffusion method was used in the test; it's also a commonly used method for determining antimicrobial susceptibility patterns **(Khalili *& al.,* 2012)**.

'Well diffusion' method (WD) was created by Magaldi in 1997 as a modification to the disc diffusion method. The procedure is similar; the discs are supplemented with dilutions of the drug placed in agar wells cut out. This enables the use and standardization of various drug concentrations for different fungal species (**Magaldi & *al.,* 2004**).

In 90 mm diameter Petri dish containing the PDA culture medium, when the medium had solidified, a central well was cut out of the agar by a sterile Pasteur Pipette and filled with the hydrosol chosen volume. Then the dishes are incubates at 24°C. Results were taken after 5 days of incubation.

1.4. Chemical analyses

The chemical analysis of essential oils and hydrosols, were carried out at the laboratories of Process Engineering department, faculty of science and technology of May 8th 1945 University under the supervision of Professor BENHAMIDA Aida.

1.4.1. pH measurement

The concentration of hydrogen ions in an ionized solution is determined using an indicator solution (such as phenolphthalein) or a pH meter. A pH of 7 denotes a neutral solution, a pH less than 7 denotes acidity, and a pH greater than 7 denotes alkalinity **(Law, 2021**).

- ***Material***
  - pH meter
  - Phenolphthalein paper
  - Sample of EOs and Hydrosols
  - Distilled water
- ***Procedure***
  - Phenolphthalein paper was used for determining pH of essential oils.
  - For the Hydrosols the pH meter was used.

1.4.2. The acid Index

Acid Index is an important indicator of vegetable oil quality. It is expressed as the amount of potassium hydroxide (KOH, in milligrams) necessary to neutralize free fatty acids contained in 1 g of oil **(Dhital, 2017)**.

- ***Material***
  - Potassium hydroxide (KOH)
  - Phenolphthalein
  - Ethanol
  - Distilled water
  - Burette
  - Flask
  - Precision balance
  - Sample of EOs and Hydrosols

- ***Procedure***

  –Preparation of the KOH solution: 11.22g of KOH is poured into a 200 ml flask containing 60 ml of distilled water; add water to the gauge line and then shake well.

  – Weigh out 0.5 g of the extracted essential oil and dissolve it in 5 ml of ethanol.

  – Add 3 drops of phenolphthalein as a color indicator.

  – Neutralize the liquid with the KOH solution (0.1N) contained in a burette.

  – Continue adding until the solution turns slightly pinkish purple.

  – Note the volume of KOH consumed.

- ***Calculation methodology***

The acid index is given by the following formula described by **Dhital (2017)**:

$$\boldsymbol{Acid\ value = \frac{(56.1 \times V \times N)}{W}}$$

Where:

V = Volume (in mL) of standard potassium hydroxide.

N = Normality of the potassium hydroxide solution.

W = Weight (in grams) of the essential oil.

56.1=Molecular weight of KOH

1.4.3. The refractive index

The refractive index of an essential oil is a unique number that designates how the oil responds and bends light. Essentially, it is a measurement that tests how the speed of light is altered when passing through the oil. Oil's refractive index can be compared to that of a reliable sample **(British Standerd Institution, 1998).**

- ***Material***

  – Refractometer

  – Samples of EOs and Hydrosols

- ***Procedure***
  - Direct the device (the refractometer) towards the light.
  - Raise the movable light prism and carefully clean both sides of visible glass.
  - Using a glass pipette, place a few drops of each obtained essential oil on the horizontal side of the reference prism without the glass pipette being made of contact with the prism.
  - Close and lock with the left knuckle.
  - Then, with the right knuckle, make a beautiful horizon between the upper light and the lower dark part.
  - Adjust the horizon with the top knuckle and with the bottom knuckle, peel off the horizon and bring it to the center of the cross.
  - Once this is done, read on the bottom scale the refractive index of each sample.

- ***Calculation methodology***

At the specified temperature T, the refractive index η_D^T is given by the equation:

$$\eta_D^T = \eta_D^{T'} + \mathbf{0.0004}(T' - T)$$

Where:

$\eta_D^{T'}$ is the reading taken at the working temperature T′at which the determination was actually made **(British Standerd Institution, 1998).**

1.5. Statistical treatment of results

EXCEL Version 2016 was used to compute the averages and standard deviations of the obtained results. 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 & Discussion

Chapter 02: Results and discussion

2.1. Yields of Essential oils

The results corresponding to the yields of essential oils of the plants used, presented in the figure **09**, show that the highest yield is obtained from *Origanum floribundum* with a value of 2.226 ± 0.064% followed by 1.813 ± 0.173 % recorded for *Mentha rotundifolia*. Lastly, *Myrtus communis* registered the lowest yield whether extracted from leaves or fruits. Moreover, it is noted that there is a huge difference between *M. communis* yields of leaves and fruits. The former extract is 1.143± 0.080 % while the later is 0.080± 0.007 %.

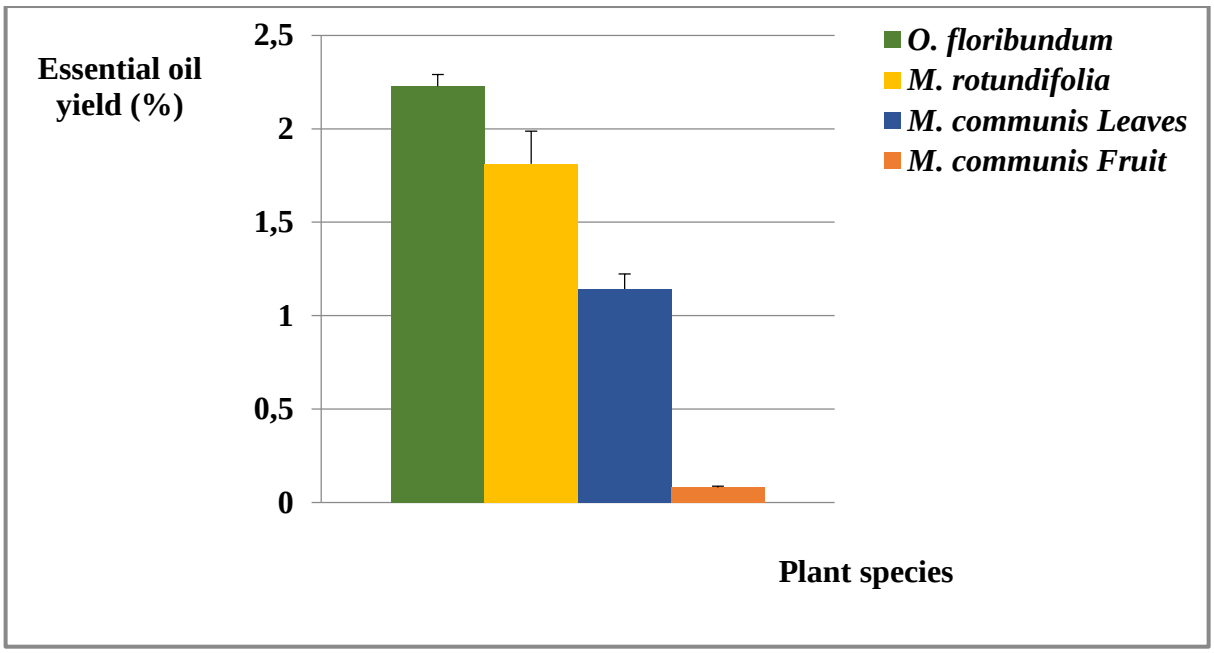

Figure 09: Yields of the essential oils ofplants used in this study

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

- **For *Myrtus communis*:**

- The extraction yield of the essential oil from *Myrtus communis* leaves is 1,143 ± 0.080 %. This is considered important compared to that obtained by **Brada & *al.* (2012)** and **Maiga (2022)**, using the same technique, the authors achieved yields of 0.3 % and 0.68% respectively.

- We also note a superiority compared to the yield obtained by **Mimica-Dukić, & *al*. (2010)**, who found an oil yield of 0.72 - 0.81%, for leaves of the same specie, collected from Montenegro, and **Allali (2017**) who had obtained a yield of 0.57 % from leaves of *M. communis* collected from Bejaia region.
- On the other hand, for*Myrtus communis* fruit (berries) our values (0.080 ± 0.007 %) are lower than those obtained by **Pereira & *al.* (2009),** which used Portuguese myrtle, and has obtained 0.11% to 0.23%, and than those obtained by **Brada & *al.* (2012)** which used myrtle from khemis-Miliana region (Aïn-Defla, Algeria), and have obtained 0.1% .
- **Mohamadi & *al.* (2021)** have obtained an average yield of 0.68 ± 0.6% from aerial parts of the myrtle collected from nineteen localities of the Algerian coast.

➢ **For *Origanum floribundum* :**

- The extraction yield of the essential oil from the dry material is 2.226 ± 0.064% which is considered important compared to that obtained by **Ferdes & Saidia (2019),** the authors have recorded a yield of 1.3%.
- While, **Achouri & *al*. (2021)** have recorded most important yield for the same plant (2.52 %), compared to our results.

➢ **For *Mentha rotundifolia*:**

- The average yield obtained on the basis of three successive extractions was 1.813±0.173%. Essential oil yields obtained for this species in Algeria are higher to results reported for the same species in Tunisia by **Riahi & *al*. (2013)** which is 1.04% to 1.26%.
- Our yields are higher than those obtained in 2009 by Derwich and his collaborators **(Derwich & *al*., 2009)**, they recorded 1.54%.
- However, our values are lower than those obtained by **Atti & Meliani (2022)**, for the same plant, collected from the same location, which are 2.95%.

Variance analysis (**Tab. 02**) has shown very high significant differences between oil's yields from the different species.

Table 02: Results of variance analysis of EOs yield for the different plant species

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Plant species	3	7.90249	2.63416	257.62	0.000***
Error	8	0.08180	0.01022		
Total	11	7.98429			

DF : degrees of freedom.
SS : Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

2.2. Organoleptic properties of the essential oils

Aspect, color and smell are among the parameters that determine the quality of oil. Thus, the organoleptic properties of our essential oil are illustrated in **table 03.**

Table 03: Organoleptic properties of essential oils extracted in this study

Origin of EO	Color	Smell	Aspect
Mentha rotundifolia	Pale yellow	Frech, strong smell	Liquid
Origanum floribundum	Yellowish to intense red	Strong smell	Liquid
Myrtus communis	Yellow very light	Strong smell	Liquid

2.3. Physical and chemical characteristics analysis

Essential oils and hydrosols are characterized by their physical properties (spectroscopy, refractive index, ...) as well as their chemical properties (acid index, pH, ...). Results obtained for our EOs and hydrosols are indicated in **table 04**.

Table 04: Physical and chemical characteristics of essential oils and hydrosols extracted in this study from different plants

Analysis / EOs	**Acid value**		**Refractive index**		**pH**	
	EO	**Hydrosol**	**EO**	**Hydrosol**	**EO**	**Hydrosol**
Mentha rotundifolia	2.244	2.244	1.481	1.330	5.3	4.5
Origanum floribundum	1.122	1.122	1.570	1.330	5.2	4.6
Myrtus communis	2.244	2.244	1.464	1.330	5.2	4.2

From the results obtained in our study we can note:

- **For *Myrtus communis* EO :**

  - The refractive index of our oils displayed a value of 1.464, which is higher than that reported by **Shaapan & *al.*(2021),** who obtained a refractive index of 1.391 of the same specie. However, this value is lower than those reported by **Allali (2017)** who found an Ri= 1.47.

  - The acid index value recorded for the EO of *M. communis* in our study (2.244) is lower than that obtained by **Allali (2017),** where the value recorded was 3.356 .

  - pH of *M. communis* EO is 5.2. This value is higher than those reported by **Maiga (2022)** who found a pH =4.9 .

- **For *Mentha rotundifolia* EO :**

  - The refractive index value of our samples (1.481) is closer to that obtained by **Atti and Meliani (2022**), and that obtained by **Benbouali (2006**), who found an Ri value of 1.541 for EO of the same specie.

  - The acid index value recorded for the EO of *M. rotundifolia*, treated in our study is 2.244.This value is lower than those reported by **Atti and Meliani (2022)** who found an Ai=2.805, and higher than that reported by **Benbouali (2006)** who found 1.78.

  - The pH of *Mentha rotundifolia* EO recorded in our study is 5.3 which indicates that our EO is acidic. This result is similar to that obtained by **Atti and Meliani (2022)** for samples of the same specie, collected from the same location.

➢ **For *Origanum floribundum* EO :**

- The refractive index value of *O. floribundum* of our sample is 1.570 which is higher to that obtained by **Boulaghmen (2012**) who found an Ri =1, 48.

- For the acid index, **Boulaghmen (2012)** found a result of 2.51, which is higher than that obtained in our study (1.122).

- pH of *O. floribundum* EO used in our study was similar to those of *M. communis* (5.2).

Hydrosols have a pH range of 4.2 to 4.5, the same results were reported by **Jakubczyk & *al*., (2021)** who indicates that hydrosols pH range of 3 to 7, indicating that they are neutral to slightly acidic.

2.4. Antifungal activity results

2.4.1. Antifungal activity of EOs

2.4.1. 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*. We will note that for *Myrtus communis*, essential oil extracted from leaves is used in the test.

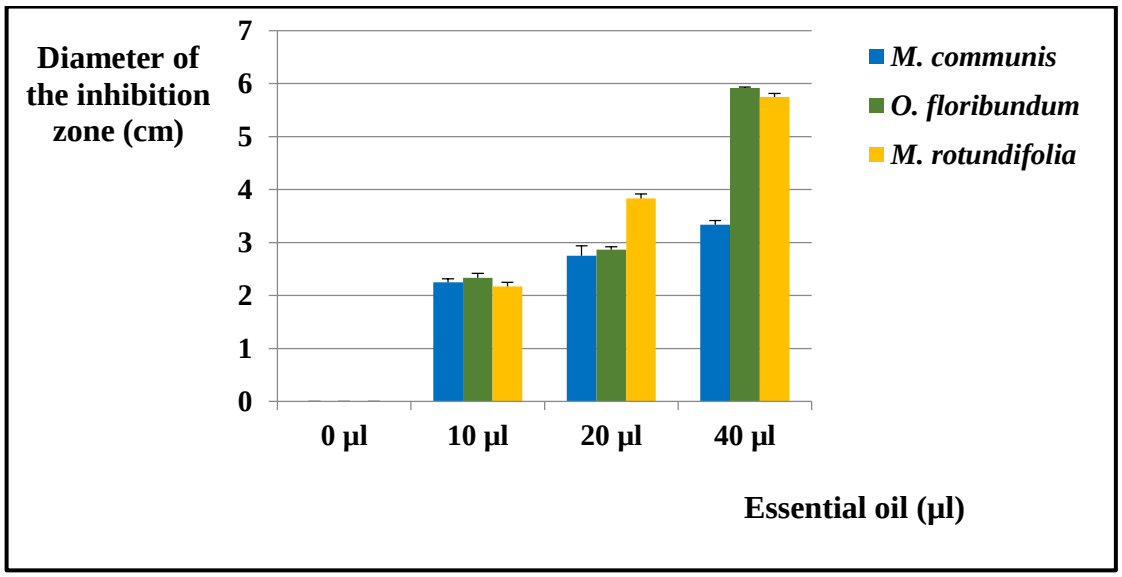

Figure 10: Diameters of growth inhibition zones of *Aspergillus niger* versus the three essential oils (*O. floribundum***,** *M. rotundifolia* and *M. Communis*).

Comparing the average inhibition zone values for *Aspergillus niger*, we can see, at 40 µL that EO of *O. floribundum* has the highest average inhibition zone with a value of 5.916 ± 0.020 cm.

M. rotundifolia has the second-highest average inhibition zone with 5.75 ± 0.0625 cm, while *M. communis* has the lowest average inhibition zone with 3.34 ± 0.08334 cm.

When considering the recorded values for *Aspergillus niger*, both *O. floribundum* and *M. rotundifolia* have higher and similar inhibition zones diameters, suggesting that their effects are higher compared to *M. communis*.

The results reported by **Touaibia (2015)** show that the pure essential oil of *Myrtus communis* exerted a very significant inhibitory effect with a diameter of the zone of inhibition of 55.27 ± 0.75 mm against *Aspergillus niger* and this activity was maintained even after 72 h.

Variances analysis (**Tabs. 05, 06 & 07)** have shown a very high differences between volumes (P= 0.000) for all oils.

Table 05 : Results of variance analysis of *M. rotundifolia* EO for *A. niger*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	53.8073	17.9358	313.06	0.000 ***
Error	8	0.4583	0.0573		
Total	11	54.2656			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F : Observed value of Fisher's F.
P : Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 06: Results of variance analysis of *O. folribundum* EO for *A. niger*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	53.3223	17.7741	451.41	0.000 ***
Error	8	0.3150	0.0394		
Total	11	53.6373			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 07: Results of variance analysis of *M. communis* EO for *A. niger*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	19.1250	6.37500	76.50	0.000 ***
Error	8	0.6667	0.08333		
Total	11	19.7917			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Dunnett tests (**Tab. 08**) have shown very high differences between all tested volumes and the control for all EOs tested.

Table 08: Dunnett test result for*Aspergillus niger* - EOs tested

Volume of EO / EO	**0 µl**	**10 µl**	**20 µl**	**40 µl**
Origanum floribundum **(Means** ± σ)	0±0	2.334 ± 0.083 ***	2.867 ± 0.053 ***	5.916 ± 0.020 ***
Mentha rotundifolia **(Means** ± σ)	0±0	2.166 ± 0.083 ***	3.833 ± 0.083 ***	5.750 ± 0.062 ***
Myrtus communis **(Means** ± σ)	0±0	2.250 ± 0.062 ***	2.750 ± 0.187 ***	3.333 ± 0.083 ***

*** :Very highly significant (α= 0.001)

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

Figure 11 presents the effects of the different EOs on the growth of *Botrytis cinerea*.

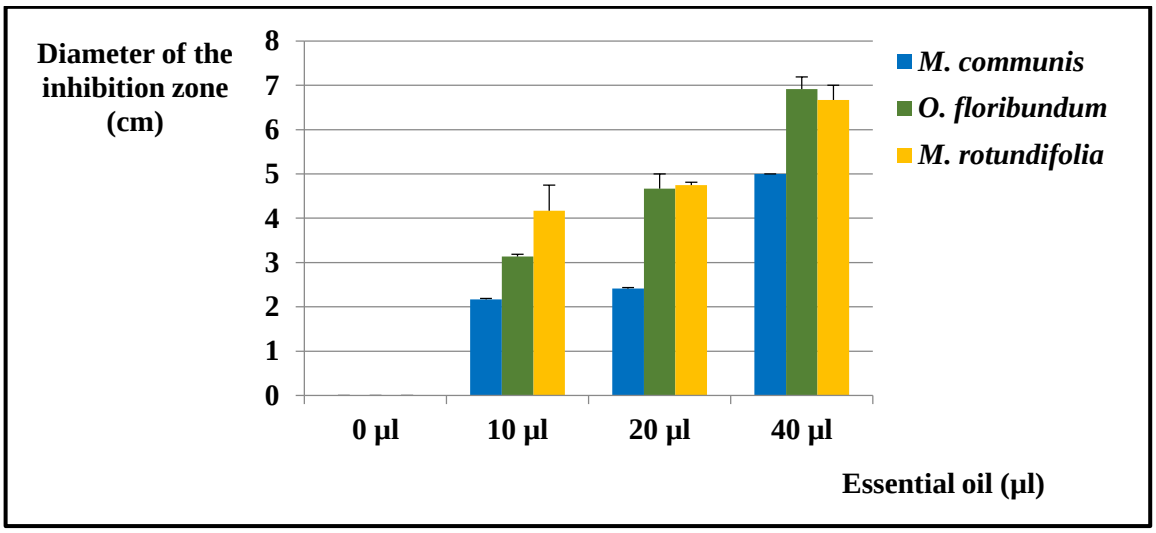

Figure 11: Diameters of growth inhibition zones of *Botrytis cinerea* versus the three essential oils (*O. floribundum*, *M. rotundifolia* and *M. Communis).*

According to the results obtained in this study (**Fig. 11 & Tab. 11**), all oils tested have inhibited the growth of *Botrytis cinerea. M. rotundifolia* seem to have an important effect on the growth ofthe fungus. The average inhibition zone was 4.166 ± 0.583 cm at 10 µl, and high comparing to *O. floribundum* (3.133 ± 0.053 cm) and *M. communis* (2.167 ± 0.020 cm). At 20 µl, an inhibition zone diameter of 4.75 ± 0.333 cm for *M. rotundifolia*, 4.667 ± 0.334 cm for *O. floribundum* and 2.417 ± 0.020 cm for *M. communis*, were noted.

Atti and Melliani (2022) have found a significant antifungal activity of *M. rotundifolia* essential oil against *Botrytis cinerea* (strain isolated from tomato fruit), the diameters of the inhibition zones are very high and exceed 8 mm for all volumes tested (5, 10, 20 & 40 µl). This suggests that this fungal species is very sensitive to this EO.

Nouiri and Touahri (2016) reported that, *Botrytis cinerea* showed sensitivity to *Mentha spicata* essential oil.

Essalhi (2022), tested the effect of different volumes of myrtle essential oil: 5, 10, 20 and 40 µL. The growth inhibition zones have an important diameters, even at low volumes of EO (5 µL) with an average of 8 mm. The inhibition of the growth of the fungus in the vicinity of the essential oil of myrtle increases proportionally with the increase in the volumes of EO. And riches 10.66 mm at the highest volume (40 µl). The author noted very significant antifungal activity against the strain of *Botrytis cinerea* used.

Results of other studies reported that EO of *M. reduntifolia* and *O. floribundum*, have an important antifungal activity against many fungi:

-Leblalta & *al*. (2020) showed that *M. rotundifolia* essential oil has significant activity against *Fusarium oxysporum,* and it completely inhibits the growth of this fungus at concentrations equal to 0.1%.

-Medjaheri and Mehadjri (2020) reported that the essential oils *Origanum* sp. have excellent antifungal properties, and at 0.025%, a total inhibition of mycelial growth for the fungus tested (*Fusarium* sp.) was observed. **Chabane (2020)** noticed a total inhibition of mycelial growth of *Fusarium* spp. isolates, under the effect of *Origanum glandulosum* essential oil.

-Boulaghman (2012), showed that the EO of *Origanum floribundum* has an important antimicrobial activity against bacterial strains of Gram+ bacteria, *Staphylococcus aureus*.

Variance analysis (**Tab. 9, 10 & 11**) have shown very high differences between volumes (P= 0.000) for all oils.

Dunnett tests (**Tab. 12**) have shown very high differences between all tested volumes and the control for the all tested oils.

Table 09: Results of variance analysis of *M. rotundifolia* EO for *B. cinerea*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	70.974	23.6580	96.65	0.000 ***
Error	8	1.958	0.2448		
Total	11	72.932			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 10: Results of variance analysis of *O. folribundum* EO for *B. cinerea*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	75.872	25.2908	153.86	0.000 ***
Error	8	1.315	0.1644		
Total	11	77.187			

DF : degrees of freedom.

SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.

P :Likelihood of highlighting significant differences.

*** p< 0.001 : Very highly significant difference.

Table 11: Results of variance analysis of *M. communis* EO for *B. cinerea*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	37.7240	12.5747	1207.17	0.000 ***
Error	8	0.0833	0.0104		
Total	11	37.8073			

DF : degrees of freedom.

SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.

P :Likelihood of highlighting significant differences.

*** p< 0.001 : Very highly significant difference.

Table 12*:* Dunnett test results for *Botrytis cinerea* **-** EOs tested

Volume of EO / EO	**0 µl**	**10 µl**	**20 µl**	**40 µl**
Origanum floribundum **(Means** ± σ)	0 ± 0	3.133 ± 0.053 ***	4.667 ± 0.334 ***	6.916 ± 0.27 ***
Mentha rotundifolia **(Means** ± σ)	0 ± 0	4.166 ± 0.583 ***	4.75 ± 0.333 ***	6.667 ± 0.333 ***
Myrtus communis **(Means** ± σ)	0 ± 0	2.167 ± 0.020 ***	2.417 ± 0.020 ***	5 ± 0 ***

*** : Very highly significant (α= 0.001)

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

The results obtained are presented in **figure 12**. All tested oils have affected the growth of *Zymoseptoria tritici*. Diameters of the inhibition zone are very important, they exceeds 2 cm for all volumes tested of all oils. The highest values were recorded for *O. floribundum*, followed by *M. rotundifolia.*

Atti and Meliani (2022) noticed an important antifungal activity of *M. rotundifolia* essential oil on *Zymoseptoria tritici*, even for low volumes of EO (5µl), where they recorded diameters of the inhibition zone, greater than 2cm, and concluded that this fungus (the same strain than that used in our study), is therefore designated as very sensitive strain to this oil, compared at *Botrytis cinerea* (isolated from tomato fruit), *Aspergillus niger* (the same strain than that used in our study) and *Fusarium roseum* (isolated from wheat).

Variance analysis (**Tab. 13, 14 & 15**) have shown very high differences between volumes (P= 0.000) for all oils.

Dunnett tests (**Tab. 16**) have shown very high differences between all tested volumes and the control for the all tested oils.

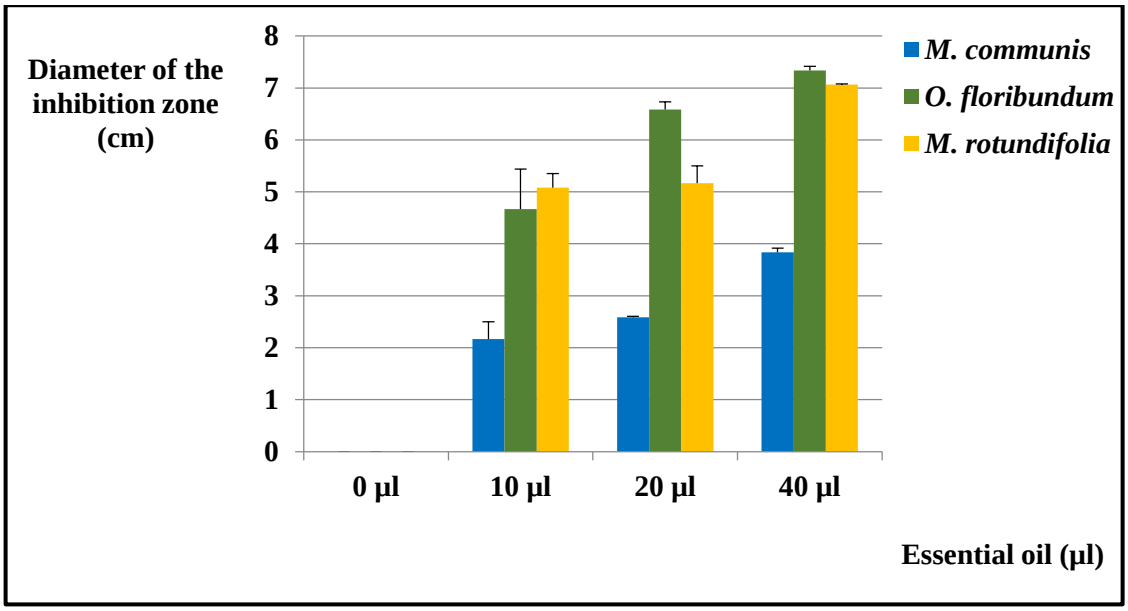

Figure 12 : Diameters of the inhibition zones of *Zymoseptoria tritici* versus the three essential oils.

Table 13: Results of variance analysis of *M. rotundifolia* EO for *Zymoseptoria tritici*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	82.517	27.5058	178.17	0.000 ***
Error	8	1.235	0.1544		
Total	11	83.752			

DF : degrees of freedom.

SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.

P :Likelihood of highlighting significant differences.

*** p< 0.001 : Very highly significant difference.

Table 14: Results of variance analysis of *O. folribundum* EO for *Zymoseptoria tritici*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	97.682	32.5608	130.24	0.000 ***
Error	8	2.000	0.2500		
Total	11	99.682			

DF : degrees of freedom.

SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.

P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference

Table 15: Results of variance analysis of *M. communis* EO for *Zymoseptoria tritici*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	22.9323	7.6441	69.89	0.000 **
Error	8	0.8750	0.1094		
Total	11	23.8073			

DF : degrees of freedom.

SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.

P :Likelihood of highlighting significant differences.
** p< 0.01 : highly significant difference

Table 16: Dunnett test results for *Zymoseptoria tritici* -EOs tested

Volume of EO / EO	0 µl	10 µl	20 µl	40 µl
Origanum floribundum **(Means** ± σ)	0 ± **0**	4.667 ± **0.770** ***	6.583 ± **0.145** ***	7.333 ± **0.083** ***
Mentha rotundifolia **(Means** ± σ)	0 ± **0**	5.083 ± **0.270** ***	5.167 ± **0.333** ***	7.067 ± **0.013** ***
Myrtus communis **(Means** ± σ)	0 ± **0**	2.167 ± **0.333** ***	2.583 ± **0.020** ***	3.833 ± **0.083** ***

***: Very highly significant (α= 0.001)

2.4.2. Antifungal activity of hydrosols

2.4.2.1. Effect of hydrosols on the growth of *Aspergillus niger*

Figure 13 show that hydrosols collected after distillation of *Myrtus communis* and *O. floribundum* have also affected growth of *Aspergillus niger*, especially *O. floribundum* for which an average of inhibition zone diameter of 1 cm has been obtained at 50 µl of hydrosol and 1.4 cm at 100 µl. However, for hydrosol of *M. rotundifolia*, no inhibition zone has been observed at all tested volumes of hydrosol, for *Aspergillus niger*.

Variance analysis (**Tab. 17**) has shown very high significant difference between volumes of hydrosol of *O. floribundum.*

Dunnett test (**Tab. 18**) have shown no significant differences between the control and volume 25 µl and very high significant differences between 50 and 100 µl of *O. floribundum* hydrosol.

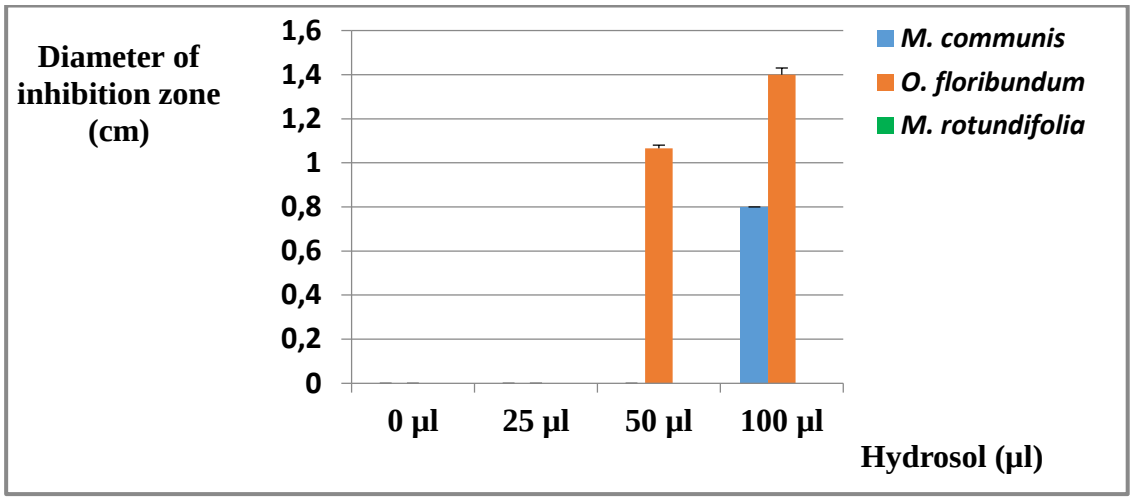

Figure 13: Diameters of growth inhibition zones of *Aspergillus niger* versus the three hydrosols

Table 17: Results of variance analysis of *O.floribundum* Hydrosol for *Aspergillus niger*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	4.73000	1.57667	145.54	0.000 ***
Error	8	0.08667	0.01083		
Total	11	4.81667			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 18: Dunnett test results for *Aspergillus niger* -Hydrosols tested

Volume of HD / Plant species	**0 µl**	**25 µl**	**50 µl**	**100 µl**
Origanum floribundum **(Means** ± σ)	0 ± **0**	0 ± **0** NS	1.066± **0.013** ***	1.400± **0.030** ***

NS:No significant (α= 0.05)
***: Very highly significant (α= 0.001)

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

Botrytis cinerea was more sensitive to the hydrosols, and it's growth was inhibited by hydrosols of the three plant species studied, notably at volumes up of 50 µl of hydrosols (**Fig. 14**). Hydrosol of *O. floribundum* have recorded the highest value of the diameter of inhibition zone, and the highest antifungal activity against *Botrytis cinerea* among the three plants. *M. rotundifolia* and *M. communis* displayed slightly lower antifungal activity.

Variance analysis have Shown very high significant differences between volumes for hydrosols of all plants (**Tab. 19, 20 & 21**).

Dunnett test (**Tab. 22**) have shown no significant difference between control and 25 µl for all hydrosols and very high significant difference between control and 50 and 100 µl for all hydrosols.

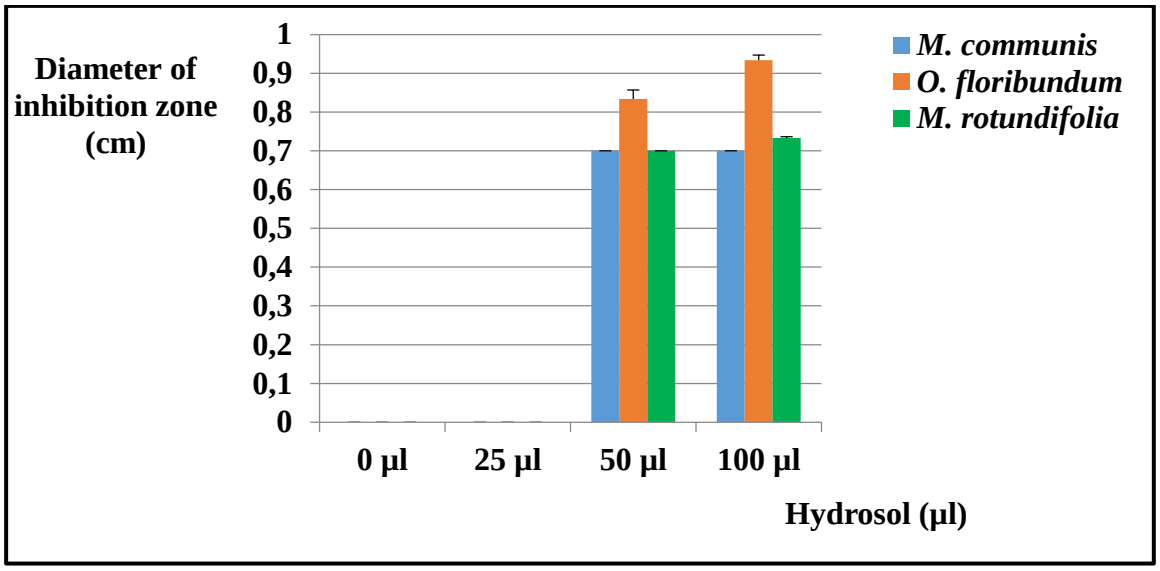

Figure 14: Diameters of inhibition zones of *Botrytis cinerea Versus* the three hydrosols

Table 19: Results of variance analysis of *M. rotundifolia* Hydrosol for *Botrytis cinerea*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	1.54250	0.514167	617.00	0.000 ***
Error	8	0.00667	0.000833		
Total	11	1.54917			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 20: Results of variance analysis of *O. folribundum* Hydrosol for *Botrytis cinerea*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	2.35583	0.785278	85.67	0.000 ***
Error	8	0.07333	0.009167		
Total	11	2.42917			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 21: Results of variance analysis of *M. Communis* Hydrosol for *Botrytis cinerea*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	1.46302	0.487675	58521.00	0.000 ***
Error	8	0.00007	0.000008		
Total	11	1.46309			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 22: Dunnett test results for *Botrytis cinerea*-Hydrosols tested

Volume of HD / Plant species	**0 µl**	**25 µl**	**50 µl**	**100 µl**
Origanum floribundum **(Means** ± σ)	0 ± 0	0.000 ± 0.000 NS	0.833± 0.023 ***	0.933± 0.013 ***
Mentha rotundifolia **(Means** ± σ)	0 ± 0	0.000± 0.000 NS	0.700± 0.000 ***	0.733± 0.003 ***
Myrtus communis **(Means** ± σ)	0 ± 0	0.000 ± 0.000 NS	0. 700± 0.000 ***	0.700 ± 0.000 ***

NS: No significant (α= 0.05)
***: Very highly significant (α= 0.001)

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

From **figure 15**, it can be seen that *O. floribundum* had the highest average antifungal activity against *Zymoseptoria tritici*, with a value of 1.500 ± 0.000 cm of zone inhibition diameter at 25 µl of hydrosol. *M. communis* and *M. rotundifolia* have shown recorded similar antifungal activity against *Z. tritici*at the tested volumes of hydrosols of the three plant species.

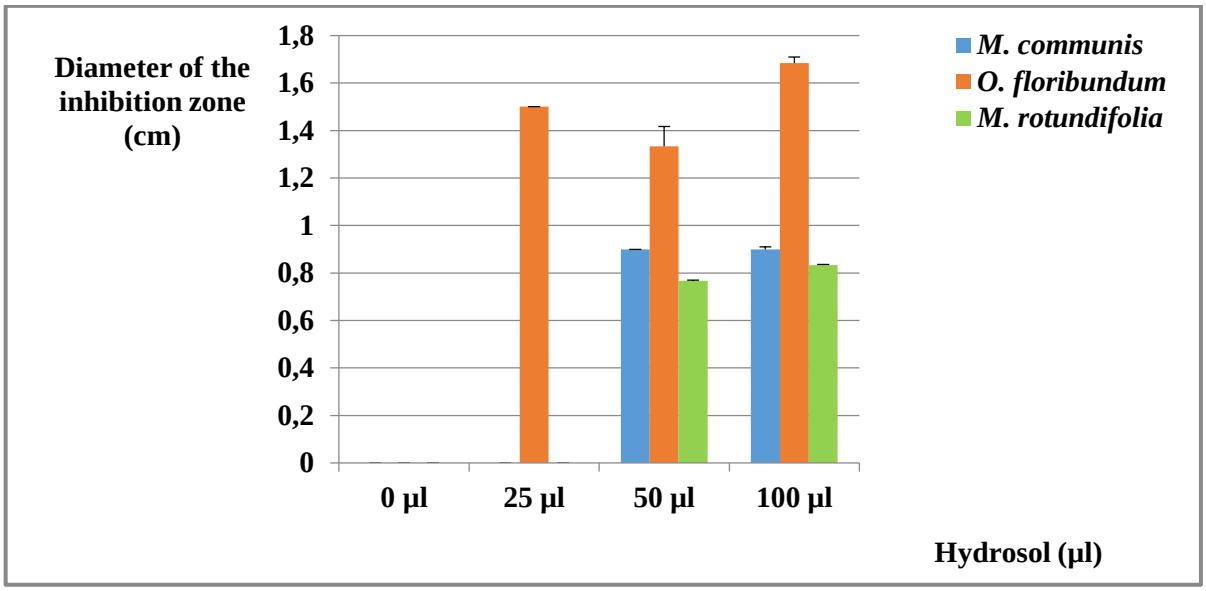

Figure 15: Diameters of inhibition zones of *Zymoseptoria tritici* versus the three hydrosols

Variance analysis (**Tabs. 23, 24& 25**) have shown very high significant differences between volumes for hydrosols of all species.

Dunnett test (**Tab. 26**) have shown no significant differences between control and 25 µl of hydrosols of *M. rotundifolia* and *M. communis*, and vey high significant differences between control and 25 µl of hydrosol of *O. floribundum*, 50 and 100 µl for all hydrosols.

Table 23: Results of variance analysis of *M. rotundifolia* Hydrosol for *Zymoseptoria tritici*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	1.92667	0.642222	385.33	0.000 ***
Error	8	0.01333	0.001667		
Total	11	1.94000			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 24: Results of variance analysis of *O. folribundum* Hydrosol for *Zymoseptoria tritici*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Volumes	3	5.2840	1.76132	64.54	0.000 ***
Error	8	0.2183	0.02729		
Total	11	5.5023			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 25 : Results of variance analysis of *M. communis* Hydrosol for *Zymoseptoria tritici*

Sources	DF	Adj SS	Adj MS	F-Vlue	P-Value
Volumes	3	2.43000	0.810000	324.00	0.000 ***
Error	8	0.02000	0.002500		
Total	11	2.45000			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 26: Dunnett test results for*Zymoseptoria tritici*-Hydrosols tested

Volume of HD / Plant species	**0 µl**	**25 µl**	**50 µl**	**100 µl**
Origanum floribundum **(Means** ± σ)	0 ± 0	1.500 ± 0.000 ***	1.333 ± 0.083 ***	1.683 ± 0.025 ***
Mentha rotundifolia **(Means** ± σ)	0 ± 0	0.000 ± 0.000 NS	0.766 ± 0.003 ***	0.833± 0.003 ***
Myrtus communis **(Means** ± σ)	0 ± 0	0.000± 0.000 NS	0.900 ± 0.000 ***	0.900 ± 0.010 ***

NS: No significant (α= 0.05)
***: Very highly significant (α= 0.001)

The efficacy of plant aqueous extracts is much lower than that of plant essential oils; however, extract preparation is much simpler **(Biniaś & *al.*, 2017).**

Zatla & *al.* (2017) found that root and 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% up 7 days of storage, demonstrating the potential of root and hydrosol extract as natural antifungal for strawberry fruits susceptible to decay caused by *B. cinerea.*

Gaspar-Pintiliescu & *al*. (2022), reported that rosemary and sage hydrosols have an antioxydant activity and an acetylcholinesterase activity inhibition; they have demonstrated many antibiotic activities (antibacterial and antifungal activity, insecticide activity), and they can be used as biopesticides.

Some industrious farmers in India collect and spray hydrosols on agricultural crops to repel insect pests and disease-causing organisms (**Rao, 2012**).

Rao (2013) reported that essential oil and undiluted hydrosol of *Satureja thtymbra* from Greece showed superior bactericidal activity against biofilm-forming bacteria. Turkish thyme, oregano, anise, cumin, black thyme, sage, rosemary and bay leaf hydrosols showed weak to moderate antibacterial activity. Indian peppermint and spearmint hydrosols showed weak to moderate antibacterial activity, while Australian lemon myrtle, Lauandula species, and Turkish rose hydrosols showed no activity on the studied strains.

According to the same author, hydrosols of Turkish *Satureja hortensis*, *Echinophora tenuifolio* and *Cumirlum cyminum* effectively inhibited the growth of phytopathogenic fungi *Alternaria citri*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *tulipe* and *Rhizoctonia solani* for 7 days. Hydrosols of anise, cumin, fennel, mint, pickling herb, oregano, savory and thyme displayed strong activity in inhibiting the mycelial growth of *Aspergillus parasiticus*. Hydrosols of lavender, thyme, chamomile, eucalyptus leaf, laurel leaf, myrtle leaf, balm leaf, heather leaf exhibited strong antifungal activity.

El-Said & Hassan (2021) demonstrated that the aqueous extract of mint leaves exhibited strong antifungal activity against fungal strains such as *Aspergillus niger* (ZID of 16-20 mm), *Candida albicans* (ZID of 21-35 mm), and *Penicillium digitatum* (ZID of 16-20 mm). The authors suggested that the hydrosol derived from mint leaves extract can be utilized in formulations for antifungal treatments or as a natural fungicide.

The aqueous extract of both mint leaves and basil leaves showed antibacterial activity against *Escherichia coli* sp. (ZID of 21-35 mm), *Enterococcus* sp. (ZID of 16-20 mm), *Bacillus* sp.

(ZID of 21-35 mm), and *Staphylococcus* sp. (ZID of 16-20 mm). The hydrosol obtained from these extracts can be incorporated into antibacterial products, such as soaps, sanitizers, or wound cleansers. Due to the antimicrobial properties of mint and basil extracts, their hydrosols can be used as natural preservatives in various products to extend their shelf life and prevent microbial growth **(El-Said & Hassan, 2021).**

Global analysis or our results, via variance analysis forall treatments tested (02 treatments of *Origanum floribundum*, 02 treatments of *Myrtus communis*, 02 treatments of *Mentha rotundifolia*), reveals a very high significant differences (p = 0.000) between treatments for the three plant pathogenic fungi studied (**Tabs. 27, 28 & 29**).

Table 27: Results of variance analysis of all treatments for *Aspergillus niger*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	5	104.9	20.973	10.33	0.000***
Error	66	134.0	2.030		
Total	71	238.8			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 28: Results of variance analysis of all treatments for *Botrytis cinerea*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	5	171.5	34.290	11.70	0.000***
Error	66	193.4	2.930		
Total	71	364.8			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.
P :Likelihood of highlighting significant differences.
*** p< 0.001 : Very highly significant difference.

Table 29: Results of variance analysis of all treatments for *Zymoseptoria tritici*

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	5	215.6	43.118	13.11	0.000***
Error	66	217.1	3.290		
Total	71	432.7			

DF : degrees of freedom.
SS :Sum of gap squares.
MS : Mean square.
F :Observed value of Fisher's F.

P :Likelihood of highlighting significant differences.

*** p< 0.001 : Very highly significant difference.

Conclusion

Conclusion

Green pesticides, also known as ecological pesticides, are pesticides derived from organic sources (plants, microorganisms,…), that are considered less harmful to human and animal health, habitats, and the ecosystem. A green revolution is defined as an increase in crop production caused by the use of new seed varieties, pesticides, new technologies, and improved management.

The geographic location of Algeria provides it with an invaluable concentration of biodiversity, and many plants are known to be an important source of bioactive molecules, with antimicrobial properties.

The main objective of the study was to characterize the physicochemical properties of essential oils and hydrosols extracted from three plants, that grow naturally in Guelma region of Algeria (*Myrtus communis*, *Origanum floribundum*, and *Mentha rotundifolia*), and evaluate their antifungal activity against three common fungal pathogens (*Aspergillus niger*, *Botrytis cinerea*, and *Zymoseptoria tritici*), responsible for black mold, gray mold, and septoria leaf blotch, respectively, which can lead to crop failures. Obtained results can be summarised as follow:

- A variation in essential oil yields among the studied plant species, has been recorded, and can be attributed to several factors: Plant species, plant parts, growth conditions…The highest yield (2.22%) was obtained from *Origanum floribundum*, followed by *Mentha rotundifolia* with 1.81%. For *Myrtus communis* EO yield, we have noted that there is a huge difference between *M. communis* yields of leaves (1.143 %) and fruits (0.080%). Very high differences were noted between species.

- Our findings provide information about the chemical properties of the essential oils and hydrosols from the three plant species. These properties can help in understanding the composition and potential uses of these oils and hydrosols in various applications. Chemical properties of the EO were nearly similar to those of hydrosols for all plant species.

- Antifungal EOs activity test, have shown very high differences between plant species and EOs volumes tested, against the studied pathogens. For all pathogens and at 40 µl, the obtained results are as follow:

- *O. floribundum* exhibited the highest antifungal activity.
- *M. rotundifolia* had the second-highest antifungal potential.
- *M. communis* had the lowest effect on all fungi.

- Antifungal hydrosols activity test, have also shown slight differences between plant species and volumes tested, against the studied pathogens. The obtained results at 100 µl of hydrosols are as follow:

- *O. floribundum* had the highest antifungal activity against all studied pathogens.
- *M. rotundifolia* had the second-highest antifungal activity against *Botrytis cinerea* and *Zymoseptoria tritici,* but it has exhibited no antifungal activity *Aspergillus niger*.
- *M. communis* hydrosol had the lowest effect on all fungi, except of *Zymoseptoria tritici* where it have recorder the second-highest activity after *O. floribundum* hydrosol.

To reiterate, these findings demonstrate that *O. floribundum* consistently displayed the highest antifungal activity against the examined fungal pathogens (*Aspergillus niger*, *Botrytis cinerea*, and *Zymoseptoria tritici*) in both essential oil and hydrosol forms. *M. rotundifolia* exhibited notable antifungal activity against *Aspergillus niger* and *Botrytis cinerea*, while *M. communis* exhibited average to lower antifungal activity across the tested fungi. These results emphasize the potential of *O. floribundum* as a potent natural antifungal agent.

In perspectives, extracts of those plants, especially *O. floribundum*, warrant further investigations into their chemical composition and potential applications in agriculture, as alternative natural products to synthetic pesticides, and intended for ecological protection of agricultural crops, low risk of developing pest resistance and sustainable management of soil and environment, and hydrosols will takes more attention in agricultural researches, in reasons as reported by **Gaspar-Pintiliescu & *al*. (2022),** valorization of by-products in the form of hydrosols represents a biotechnological advantage, because, unlike essential oils, hydrosols contain low amount of volatile compounds, and this could be a plus in terms of reduced toxicity when used as natural pesticides.

Abstract

Abstract

Our study aims to evaluate properties and antifungal activity of essential oils and hydrosols extracted from *Myrtus communis, Origanum floribundum* and *Mentha rotundifolia*, which grow spontaneously in the Guelma region (Algeria). These plants are tested against *Aspergillus niger, Botrytis cinerea,* and *Zymoseptoria tritici.* Hydrodistillation technique is used to extract the essential oils, employing a *Clevenger*-type apparatus. The highest yield of EO is obtained from *Origanum floribundum*, followed by *Mentha rotundifolia*. Antifungal activity is conducted by direct confrontation, using disc diffusion method for oils and well diffusion method for hydrosols tests. Chemical analyses have shown low differences between oil and hydrosol characteristics, of the same plant specie. In this study, we noticed very satisfactory antifungal activity against studied pathogens at tested volumes, for EOs and hydrosols of all plant species, especially for *O. floribundum.* These findings contribute to the understanding of the antifungal potential of these essential oils and hydrosols, highlighting their potential as natural alternatives to control plant pathogens, reducing crop losses and protecting environment.

Keywords*:* *Myrtus communis, Origano floribundum, Mentha rotundifolia,* Plant pathogens, Essential oil, Hydrosol, Antifungal activity.

Résumé

Notre étude vise à évaluer les propriétés et l'activité antifongique des huiles essentielles et des hydrolats extraits de *Myrtus communis*, *Origanum floribundum* et *Mentha rotundifolia*, qui poussent spontanément dans la région de Guelma (Algérie). Ces plantes sont testées contre *Aspergillus niger*, *Botrytis cinerea* et *Zymoseptoria tritici*. La technique d'hydrodistillation est utilisée pour extraire les huiles essentielles, en utilisant un appareil de type Clevenger. Le rendement le plus élevé en huile essentielle est obtenu à partir *d'Origanum floribundum*, suivi de *Mentha rotundifolia*. L'activité antifongique est réalisée par confrontation directe, en utilisant la méthode de diffusion sur disque pour les tests d'huiles et la méthode de diffusion dans un puits pour les hydrolats. Les analyses chimiques ont montré de faibles différences entre les caractéristiques des huiles et des hydrolats de la même espèce végétale. Dans cette étude, nous avons constaté une activité antifongique très satisfaisante contre les agents pathogènes étudiés aux volumes testés, pour les huiles essentielles et les hydrolats de toutes les espèces végétales, en particulier pour *O. floribundum.* Ces résultats contribuent à la compréhension du potentiel antifongique de ces huiles essentielles et hydrolats, mettant en évidence leur potentiel en tant qu'alternatives naturelles pour contrôler les agents pathogènes des plantes, réduire les pertes de récolte et protéger l'environnement.

Mots-clés : *Myrtus communis*, *Origanum floribundum*, *Mentha rotundifolia*, agents pathogènes des plantes, huile essentielle, hydrolat, activité antifongique.

ملخص

تهدف دراستنا إلى تقييم الخصائص والنشاط المضاد للفطريات للزيوت العطرية والمائيات المستخلصة من Myrtus communis، Origanum floribundum و Mentha rotundifolia ، التي تنمو بشكل عفوي في منطقة قالمة (الجزائر). يتم اختبار هذه النباتات ضد أنواع فطرية مثل Aspergillus niger و Botrytis cinerea و Zymoseptoria tritici. يتم استخدام تقنية التقطير المائي لاستخلاص الزيوت العطرية باستخدام جهاز من نوع Clevenger يتم الحصول على أعلى عائد للزيت العطري من Origanum floribundum، تليها Mentha rotundifolia. يتم إجراء النشاط المضاد للفطريات عن طريق المواجهة المباشرة، باستخدام طريقة انتشار الأقراص لاختبار الزيوت العطرية وطريقة انتشار الآبار لاختبار مستخلص مائي. أظهر التحليل الكيميائي اختلافات ضئيلة بين الخصائص العطرية والمائية لنفس النبات. في هذه الدراسة، لاحظنا نشاطًا مضادًا للفطريات مرضيًا جدًا ضد الأمراض المعروفة في الحجم المختبر، بالنسبة للزيوت العطرية والمائيات لجميع أنواع النباتات، وخاصةً بالنسبة لـ O. floribundum. تساهم هذه النتائج في فهم القدرة المضادة للفطريات لهذه الزيوت العطرية والمائيات، مما يسلط الضوء على إمكانية استخدامها كبدائل طبيعية للسيطرة على الأمراض المعروفة للنباتات، وتقليل الخسائر الزراعية وحماية البيئة.

الكلمات لمفتاحية : Myrtus communis ، Origanum floribundum، Mentha rotundifolia، مسببات الأمراض النباتية ، زيت أساسية ، ، مستخلصات مائية، نشاط مضاد للفطريات.

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Abstract

Our study aims to evaluate properties and antifungal activity of essential oils and hydrosols extracted from *Myrtus communis*, *Origanum floribundum* and *Mentha rotundifolia*, which grow spontaneously in the Guelma region (Algeria). These plants are tested against *Aspergillus niger*, *Botrytis cinerea*, and *Zymoseptoria tritici*. Hydrodistillation technique is used to extract the essential oils, employing a Clevenger-type apparatus. The highest yield of EO is obtained from *Origanum floribundum*, followed by *Mentha rotundifolia*. Antifungal activity is conducted by direct confrontation, using disc diffusion method for oils and well diffusion method for hydrosols tests. Chemical analyses have shown low differences between oil and hydrosol characteristics, of the same plant specie. In this study, we noticed very satisfactory antifungal activity against studied pathogens at tested volumes, for EOs and hydrosols of all plant species, especially for *O. floribundum*. These findings contribute to the understanding of the antifungal potential of these essential oils and hydrosols, highlighting their potential as natural alternatives to control plant pathogens, reducing crop losses and protecting environment.

Keywords: *Myrtus communis*, *Origano floribundum*, *Mentha rotundifolia*, Plant pathogens, Essential oil, Hydrosol, Antifungal activity

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Résumé

Notre étude vise à évaluer les propriétés et l'activité antifongique des huiles essentielles et des hydrolats extraites de *Myrtus communis*, *Origanum floribundum* et *Mentha rotundifolia*, qui poussent spontanément dans la région de Guelma (Algérie). Ces plantes sont testées contre *Aspergillus niger*, *Botrytis cinerea* et *Zymoseptoria tritici*. La technique d'hydrodistillation est utilisée pour extraire les huiles essentielles, en utilisant un appareil de type Clevenger. Le rendement le plus élevé en huile essentielle est obtenu à partir *d'Origanum floribundum*, suivi de *Mentha rotundifolia*. L'activité antifongique est réalisée par confrontation directe, en utilisant la méthode de diffusion sur disque pour les tests d'huiles et la méthode de diffusion dans un puits pour les hydrolats. Les analyses chimiques ont montré de faibles différences entre les caractéristiques des huiles et des hydrolats de la même espèce végétale. Dans cette étude, nous avons constaté une activité antifongique très satisfaisante contre les agents pathogènes étudiés aux volumes testés, pour les huiles essentielles et les hydrolats de toutes les espèces végétales, en particulier pour *O. floribundum.* Ces résultats contribuent à la compréhension du potentiel antifongique de ces huiles essentielles et hydrolats, mettant en évidence leur potentiel en tant qu'alternatives naturelles pour contrôler les agents pathogènes des plantes, réduire les pertes de récolte et protéger l'environnement.

Mots-clés : *Myrtus communis*, *Origanum floribundum*, *Mentha rotundifolia*, agents pathogènes des plantes, huile essentielle, hydrolat, activité antifongique.

ملخص

تهدف دراستنا إلى تقييم الخصائص والنشاط المضاد للفطريات للزيوت العطرية والمائيات المستخلصة من*Myrtus communis*، *Origanum floribundum* و*Mentha rotundifolia* ، التي تنمو بشكل عفوي في منطقة قالمة (الجزائر). يتم اختبار هذه النباتات ضد أنواع فطرية مثل *Aspergillus niger* و *Botrytis cinerea* و *Zymoseptoria tritici*. يتم استخدام تقنية التقطير المائي لاستخلاص الزيوت العطرية باستخدام جهاز من نوع Clevenger يتم الحصول على أعلى عائد للزيت العطري من*Origanum floribundum* ، تليها *Mentha rotundifolia*. يتم إجراء النشاط المضاد للفطريات عن طريق المواجهة المباشرة، باستخدام طريقة انتشار الأقراص لاختبار الزيوت العطرية وطريقة انتشار الآبار لاختبار مستخلص مائي. أظهر التحليل الكيميائي اختلافات ضئيلة بين الخصائص العطرية والمائية لنفس النبات. في هذه الدراسة، لاحظنا نشاطًا مضادًا للفطريات مرضيًا جدًا ضد الأمراض المعروفة في الحجم المختبر، بالنسبة للزيوت العطرية والمائيات لجميع أنواع النباتات، وخاصةً بالنسبة لـ *O. floribundum*. تساهم هذه النتائج في فهم القدرة المضادة للفطريات لهذه الزيوت العطرية والمائيات، مما يسلط الضوء على إمكانية استخدامها كبدائل طبيعية للسيطرة على الأمراض المعروفة للنباتات، وتقليل الخسائر الزراعية وحماية البيئة.

الكلمات لمفتاحية : *Myrtus communis* ، *Origanum floribundum*، *Mentha rotundifolia*، مسببات الأمراض النباتية ، زيت أساسية ، ، مستخلصات مائية، نشاط مضاد للفطريات.