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Potential of some medicinal and aromatic plants grown in Algeria as biopesticides

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General Introduction

General introduction

The nutritional health and well-being of humans are entirely dependent on plant foods. Plants are critical components of the dietary food chain in that they provide almost all essential mineral and organic nutrients to humans either directly, or indirectly when plants are consumed by animals, which are then consumed by humans. Because plants are autotrophic, they can acquire elemental compounds and convert these into the building blocks needed to make all complex macromolecules necessary to support plant growth and reproduction. Humans, on the other hand, require many of the same mineral nutrients as plants, but have additional requirements for various complex organic molecules. With the exception of vitamins B12 and D, a plant-based food supply can ensure the adequate nutrition of humans at all stages of life **(Grusak & Dellapenna, 1999).**

Microbes have been interacting with plants for hundreds of millions of years. Plant–microbe interactions have taken various forms, such as commensalism (microbes living off compounds naturally released by plants), endophytism (microbes living inside plants without affecting the host's fitness), symbiosis (the interacting organisms together are fitter than the separate organisms) and parasitism (reduced fitness of the plant for the benefit of the microbe). In the case of parasitism, the plant and microorganism have competing interests (**Takken & Rep, 2010**).

Plant diseases cause major production and economic losses in agriculture and forestry. It is estimated that the crop losses due to plant pathogens in United Stated result in about 33 billion dollars every year (**Sankaran & *al*., 2010**).

According to **Bhandari & *al*. (2021**) fungi are the most damaging agents in agriculture sector among all the other groups of microorganisms. Losses suffered by crops due to microbial disease are 16%, out of which 70-80% losses were caused by fungi. *Fusarium* sp., *Aspergillus sp*., *Botrytis* sp., *Puccinia* sp. *Erysiphe* sp., and many others, are among the plant pathogenic fungi of economic importance.

Fusarium is a genus of filamentous ascomycete fungi that includes many toxin-producing plant pathogens of agricultural importance. Collectively, *Fusarium* diseases include wilts, blights, rots, and cankers of many horticultural, field, ornamental, and forest crops in both agricultural and natural ecosystems. Fusaria also produce a diverse array of toxic secondary metabolites (mycotoxins), such as trichothecenes and fumonisins that can contaminate agricultural products, making them unsuitable for food or feed (**Ma & *al.*, 2013**).

Fusarium species cause a huge range of diseases on an extraordinary range of host plants. The fungus can be soilborne, airborne, or carried in plant residue, and can be recovered from any part of a plant from the deepest root to the highest flower (**Summerell & *al.*, 2003**). *F. oxysporum* strains can cause vascular wilt or root rot (**Fig. 01**) in over 100 plant species, including banana, bulb flowers, cucumber, date palm, melon and tomato (**Lievens & *al.*, 2008**).

Figure 01: Symptoms of *Fusarium* on tomato stem **(a) [1]**, and fruits **(b) [2]**.

Aspergillus niger, the primary cause of the "black mold" disease is considered the most prevalent contaminant in stored food (**Fig. 02**). It is responsible for the postharvest decay of fresh fruits, grains, and crops across the globe **(Lima & *al.*, 2019).** Reports indicate that *Aspergillus niger* can function as a plant pathogen, leading to significant economic losses by causing rotting in various fruits, vegetables, and food products **(Gautam & *al.*, 2011).**

Figure 02: Black mold caused by *Aspergillus niger* on onion **[3]**.

The *Botrytis* species are polyphagia parasites which are able to live either as saprophytes, feeding off decomposing organic material, or as parasites or semi-parasites and can even alternate between periods of each type of behavior. These types of parasites produce toxins as well as wall-degrading enzymes and have a wide host range (**Aleu & Collado, 2000**). *Botrytis cinerea,* causal agent of grey mold (**Fig. 02**), is an airborne plant pathogen with a necrotrophic lifestyle attacking over 200 crop hosts worldwide (**Williamson & *al*., 2007**).

Figure 03: Symptoms of *Botrytis cinerea* on tomato (**a**) **[4]**, and strawberry fruits (**b**) **[5]**.

Septoria represents a genus of plant pathogenic fungi with a wide geographic distribution, commonly associated with leaf spots and stem cankers of a broad range of plant hosts. A total of 47 clades or genera were resolved, leading to the introduction of 14 new genera, 36 new species, and 19 new combinations (Quaedvlieg & *al*., 2013). *Zymoseptoria tritici* (**Fig. 03**), the causal agent of Septoria tritici blotch (STB), is one of the most devastating diseases of wheat (**Gurr & Fones, 2015**). This plant pathogenic fungus infects wheat worldwide and can cause up to 50% yield losses per year in wheat-growing countries (**Fones & Gurr, 2015**).

Figure 04: Symptoms of Septoria tritici bloch on wheat **[6]**

Crop protection plays a vital role in food production worldwide. However, there are concerns associated with the agricultural use of fungicides **(Kamel & *al*., 2019)**. The extensive and indiscriminate application of pesticides in agriculture has led to numerous environmental issues, including water, soil, and food contamination, poisoning of farmers, harm to non-target organisms, and the development of resistance in pests, pathogens, and weeds (**Stangarlini & *al*., 2011**). For *Botrytis* species there are fungicides for its control, but many classes of fungicides have failed due to its genetic plasticity (**Williamson & *al*., 2007**).

According to **Arshad & *al*. (2014**), it has been projected that in each year, approximately 2.5 million tons pesticides have been used on crops and the pesticides caused global spoil of about $100 billion per year. This is because of the non-biodegradable properties and high toxicity of pesticides in the water resources, residues in soil, and crops which influence public health. As such, there is a growing need to search the biodegradable and new highly selective pesticides to solve the problem of long-term toxicity to mammals, and the environment friendly pesticides and develop techniques that can be used to decrease the pesticidal use though maintaining crop yields.

Efforts to mitigate these adverse effects involve exploring alternative methods of plant disease management, such as biological control, induced resistance, and the use of natural products like extracts and essential oils from medicinal plants, as well as extracts from basidiomycetes (**Stangarlin & *al*., 2011**).

In nature, essential oils play an important role in plant protection, as antibacterials, antivirals, antifungals, insecticides and also against herbivores, they are used in embalment, preservation of foods and as antimicrobial remedies. Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional pharmacopoeia. Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. They are liquid, limpid and rarely colored, lipid soluble and soluble in organic solvents with a generally lower density than that of water. They can be synthesized by all plant organs, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes (**bakkali & *al.*, 2008).**

With an area of 2,381,741 km^2, Algeria is the largest riparian country of the Mediterranean. It is recognized by its diversity in medicinal and aromatic plants, and they have various popular uses throughout the different regions of the country. There is approximately 1779 species, among them, 35 species extremely rare and 168 endemic species. These plants are certainly abundant, but geographically dispersed and have low yield potential **(FAO (2012) *in* Sahi & *al.*, 2016).**

According to **Sahraoui & *al*. (2007),** Algeria like all the Maghreb countries, due to its climate and its fertile land, has a very rich flora, in particular in medicinal and aromatic plants. Several of these plants are sources of bioactive substances that are still little used or not exploited.

A medicinal plant according to the World Health Organization is any plant which, as whole or in one or more of its organs, either contains such substances that can be used for synthesis of useful drugs or contains such substances with medicinal properties that can be used directly for therapeutic purposes Such plants as whole or their parts such as roots, stem, leaves, stem barks, fruits or seed can be applied in control or treatment of a disease (**Ahad & *al*. (2021)**.

Medicinal and aromatic plants are important mainly because they contain plant secondary metabolites (such as essential oils, alkaloids, glygosides, saponins, tannins, vitamins, and other bioactives). Plant secondary metabolites are differentiated from plant primary metabolites of photosynthesis and respiration that are directly involved in growth and development of plants **(**Zheljazkov & Craker (2015) *in* **Zheljazkov & Cantrell, 2016**).

According to **Xiong & *al*. (2021**), plant essential oils are viewed as promising biological antifungal agents promoting a safe strategy for the control of postharvest disease of fresh produce. Moreover, essential oils are widely used in agriculture and food industry because they have number of beneficial functions which include maintenance of sterile environments, antiviral, antibacterial, antifungal and antioxidants. Essential oils usage also conforms to consumers' expectations for natural foods.

Lamiaceae family has cosmopolitan distribution. Many members of this family are useful economically for medicinal, culinary, ornamental and various commercial utilizations (**Al –Naser & Al-Abrass, 2014**). The genus *Lavandula* (**Fig. 04**), consists of about 30–32 species, distributed from the North Atlantic Islands (Macaronesia) to the Mediterranean Basin, North Africa, the Middle East, tropical NE Africa, and India regions (**Saadi & *al*., 2016**). Previous studies on the essential oils of many Lamiaceae show that, these plants have a broad range of biological activities, notably their antimicrobial potency. Lavender (*Lavandula officinalis* L.), a member of the Lamiaceae (Labiatae) family, native to southern Europe and the Mediterranean area, grows in full sun on dry, well-drained, stony calcareous soils. Lavender oils contain more than 100 compounds, with the two major constituents being linalool and linalylacetate (**Al –Naser & Al- Abrass, 2014**). Lavender essential oils have been found to possess diverse activities including medicinal, preservative, insecticidal, antimicrobial and antifungal properties (**Erland & *al*., 2016**). It is a potential broad spectrum antimicrobial substance with a strong inhibitory effect on various phytopathogenic micro-organisms (**Xiong & *al*., 2021**).

Figure 05: Lavender plant (*L. angustifolia*) **[7]**.

Due to their content in essential oils (EOs), *Lavandula* species are one of the most useful aromatic and medicinal plants with a great economic value for pharmaceutical, food, and flavor industries, cosmetics, perfumery, and aromatherapy **(Aprotosoaie & *al.*, 2017).**

Lavender essential oils have been used in complementary medicines, in cosmetics, or as food additives for centuries, because these oils have been traditionally regarded as antibacterial and antifungal agents **(Park & *al.*, 2019).**

The genus *Origanum* (Lamiacae) is also widespread in the Mediterranean area. Oregano plants (**Fig. 05**) are largely used in the flavoring of food products and in perfumer. *Origanum* species have continuously been studied for their essential oil composition as potential natural sources of antimicrobial and antioxidant agents (**Berrehal & *al.*, 2010**). The leaves, the flowers and the stems of Oregano have a strong anticeptic and antimicrobial activity mainly due to the basic constituents including carvacrol, thymol, γ-terpinene and p-cymene (**Wogiatzi & *al.*, 2009**).

Figure 06: Greek oregano plant (*Origanum vulgare*) **[8].**

Also, among the Lamiaceae family, the genus *Thymus* is one of the most studied genera, due to the use of these plants as a remedy in traditional medicine and as a condiment, mainly in the Mediterranean zone. High bioactivity (e.g., antioxidant, anti-inflammatory, antiproliferative, and antimicrobial effects) is linked to the considerable content in phytochemicals of different *Thymus* species. Volatile compounds are extracted in essential oils (EOs), while nonvolatile compounds are found in alcoholic and aqueous extracts obtained by maceration, decoction, or infusion **(Leal & *al.*, 2017).**

Thymus spp. contains a number of chemicals including thymol, γ-terpinene, ρ-cymene, linalool, myrcene, α-pinene, carvacrol, α –thujene, and the eugenol which is a powerful antibacterial agent (**Al-shahrani & *al.*, 2017**). Several studies have shown that thyme oils, particularly those of *Thymus vulgaris* (**Fig. 06)** and *Thymus zygis* possess antimicrobial activity (**Pinto & *al*., 2006**).

Figure 07: Thym plant (*T. vulgaris*) **[9]**

Ruta, the wealthiest genus within the Rutaceae family, originates from the Mediterranean region and has a long history in traditional medicine, is now extensively cultivated worldwide for its medicinal properties. In Algeria, there are five species of the genus *Ruta*: *Ruta montana*, *Ruta chalepensis*, *Ruta graveolens* (**Fig. 07**), *Ruta angustifolia*, and *Ruta tuberculata* **(kambouche & *al*., 2007).**

Figure 08: *Ruta graveolens* plant **[10]**

Previous research has demonstrated that *R. montana* is a rich source of various bioactive compounds, including alkaloids, coumarins, flavonoids, tannins, and essential oils. These compounds have been found to exhibit a range of biological activities, including antifungal, antibacterial, antioxidant, insecticidal, and larvicidal properties **(Mohammedi & *al*., 2019).**

This study focused on evaluating the potential of essential oils derived from three aromatic plants grown in Guelma region of northeastern Algeria, as botanical pesticides. We have examined the yield and antifungal activity of the essential oils extracted from the aerial parts of *Thymus vulgare*, *Lavandula stoechas*, and *Ruta montana* against two plant pathogenic fungi *Aspergillus niger* and *Zymoseptoria tritici*.

The primary objective of this study is to explore the possibility of using these natural compounds as botanical pesticides. We aimed in this study to mitigate the negative impact on the environment, non-target organisms and human health, due to their residual effects, and reducing the reliance on synthetic pesticides.

Material and Methods

Chapter 01: Material and methods

The objective of our work is to evaluate the yields and the antifungal activities of essentials oils extracted from some medicinal and aromatic plants grown in Guelma region (North-East of Algeria)

1.1. Plant material

Our research focused on the aerial parts of three aromatic and medicinal plant species, collected from Guelma region: *Thymus vulgaris*, *Ruta montana* and *Lavendula stoekas*. The origins of these plants is indicated in **Table 01**.

Table 01: Origins and characters of plant material used

Species	**Family**	**Date of collection**	**Location**	**Part used**
Thymus vulgaris	Lamiaceae	March 2024	Aïn larbi (Guelma) – Altitude 36°15'57''North – Longitude 7°23'52''East	Leaves
Ruta montana	Rutaceae	March 2024	Aïn larbi (Guelma) – Altitude 36°15'57''North – Longitude 7°23'52''East	Leaves
Lavendula stoekas ssp. *pedunculata*	Lamiaceae	April 2021	Bouati mahmoud (Guelma) – Altitude36°35'32''North – Longitude 7°19'41''East	Leaves and flowers

The plant species were selected based on the following factors:

- Their extensive use in traditional medicine, such as in the preparation of herbal teas and other products, for treating human microbial diseases and infections.
- Their wide availability as natural botanical resources across the country, specifically in eastern Algeria.
- Lack of research on the biopesticide properties of these plant species' essential oils, especially, their antifungal capabilities.

1.1.1. Presentation of Guelma region, origin of plant material

Guelma, origin of the plant species used, is located 60 km far from Annaba, in the north of Algeria (**Fig. 08**). It covers an area of 3686.84 km^2 and is located 279 m above sea level. It is located halfway between the north, the Highlands and the south of the country**.** Geographic coordinate of Guelma are : Latitude of 36°46′ North and Longitude of 7°28′East (**Haddad & *al*., 2015**).

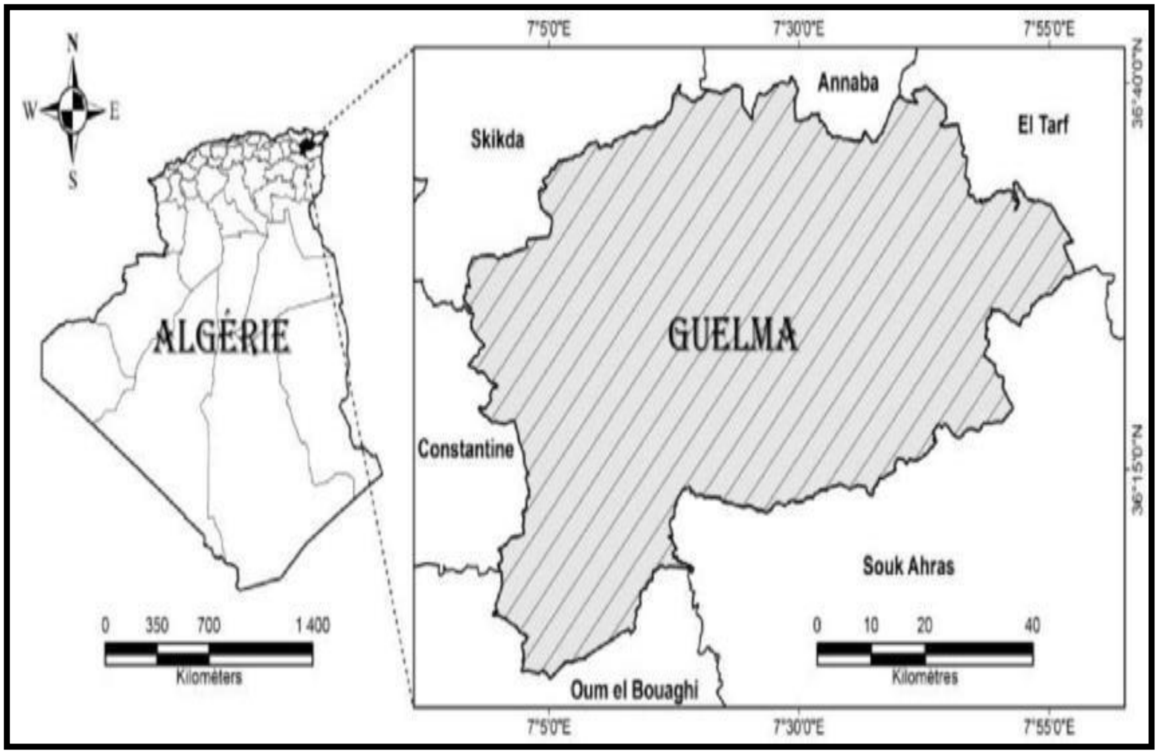

Figure 09: Geographic situation of Guelma region **(Hamed & *al*., 2019)**

Guelma is characterized by a sub-humid climate in the center and the North and semi-arid towards the South. This climate is mild and rainy in winter and hot in summer. The temperature which varies from 4° C in winter to more than 35° C in summer is on average of 17.3° C. **[11].**

Guelma is characterized by diverse relief, where forest cover (12%), and agriculture area (35%). It has an agro-sylvo-pastoral vocation. The region's geography is diversified, and it is being the largest and most significant in terms of forest cover **(Beldjazia, 2016).**

According to **Abdraman & *al*. (2014)**, Guelma region is characterized by a diverse range of formations, spanning from the Quaternary to the Triassic periods. This diversity is reflected in the region's varied lithology, which includes: Alluvium (sand, gravel, pebbles, etc.), Sandstone, Marl, Clay and Limestone.

1.1.2. Samples treatment

After gathering, the plants were thoroughly cleaned and left to dry in a well-ventilated space at room temperature for 7 to 15 days, varying depending on the species. Once dried, the samples (leaves for *Thymus* vulgare, and *Ruta montana*, leaves and flowers for Lavender *Lavendula stoekas*), were separated from the plant's other parts and stored in clean bags until ready to be used.

1.1.3. Extraction of essential oils

Essential oils of the plants were obtained through hydrodistillation using a Clevenger-type apparatus **(Fig. 09)**, in the laboratories of the Faculty of Natural and Life Sciences and Earth and Universe Sciences at 8th May 1945 University in Guelma.

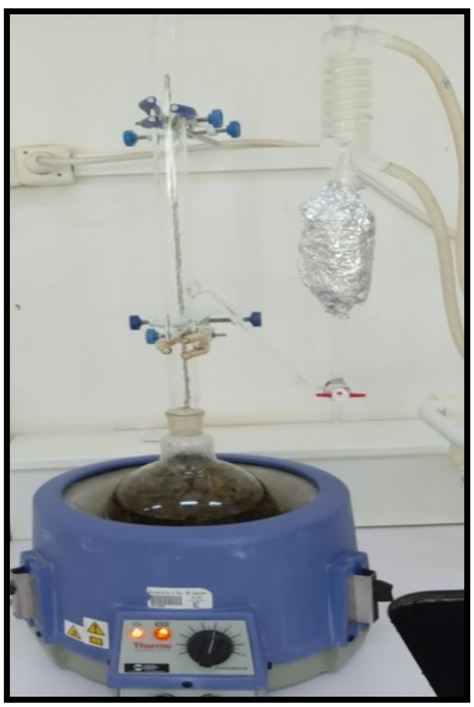

Figure 10: Clevenger apparatus used for essential oils extraction

Hydrodistillation is a common method for extracting bioactive compounds, particularly essential oils. In the Clevenger apparatus, the sample is heated while hydrated, causing volatile components to vaporize. The method results in two layers - an aqueous layer and an oil-rich layer. The oil can then be further separated. Hydrodistillation is advantageous because it does not require organic solvents, making it a preferred choice when extraction costs are a concern. The hydrodistillation process involves three main physicochemical mechanisms: hydrodiffusion, hydrolysis, and heat decomposition **(Fagbeni & *al.*, 2021).**

1.1.4. Preservation of the essential oils obtained

The essential oils obtained from various plant species were individually stored in sterile, clean containers and kept in a cool location (Freezer).

1.1.5. Determination of yield's essential oils

The essential oil yield refers to the quantity of essential oil extracted from a given amount of dried plant material. It is typically expressed as a percentage and calculated using the formula:

Essential oil yield (%) = (Mass of extracted essential oil / Mass of dried plant material) × 100

This formula, as reported by **Samadi & *al.* (2020)**., provides a standardized way to quantify the efficiency of the essential oil extraction process and allows for comparison between different plant samples or extraction methods.

1.2. Fungal material

This study is focused on two plant pathogenic fungi species:

- *Zymoseptoria tritici*: causal agent of Septoria tritici blotch of wheat (STB)
- *Aspergillus niger* : Causal agent of black mold on onion.

1.2.1. Origins of fungi's samples

Zymoseptoria tritici was obtained from the leaves of common wheat exhibiting symptoms of septoria tritici blotch, and *Aspergillus niger* is isolated from onion vegetables.

1.2.2. Cultivation and preservation of strains

The fungal strains were cultured on PDA medium (Potato Dextrose Agar), incubated for 5 days (for *Aspergillus niger*) to 7 days (for *Zymoseptoria tritici*) at 24°C, and subsequently kept at 4°C for later 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

– Pasteur Pipettes

1.3.2. Preparation of spore suspensions

Sporal suspensions for the fungal strains were created from young cultures, 5 days old for *Aspergillus niger*, and 7 days old for *Zymoseptoria tritici*. The spores were collected by scraping in a 15 mL sterile plastic tapered tubes filled with 0.9 % physiological water solution and 2 drops of Tween 80. The concentrations were adjusted to the specific values mentioned in the literature for the two pathogens:

— For *Aspergillus niger*, a sporal concentration of 10^6 spores/mL (**Li & *al*., 2019**), is used.

— For *Zymoseptoria tritici*, a sporal concentration of 3 × 107 spores /mL **(Perelló & *al*., 2013)** is used.

1.3.3. Fungal strains-Essential Oils Susceptibility test

To assess the susceptibility of the fungal strains at the essential oils studied, we have use varying volumes of pure essential oils: 2.5µL, 5µL, 10 µL, 20 µL, 40 µL, and a negative control (0 µL) treated with sterilized distilled water**.**

The disk diffusion method was used to assess the antifungal activity of essential oils (EOs). This direct confrontation technique involves the diffusion of EOs through 6 mm diameter Whatman paper discs placed in the center of Petri dishes containing potato dextrose agar (PDA) inoculated with the target fungal strains. Specifically, a 500 µL spore suspension, prepared the same day, from fresh culture, was spread evenly over the PDA medium in each 90 mm diameter Petri dish. After allowing the inoculum to dry for a few minutes, a 6 mm Whatman paper disc was placed in the center of each dish and soaked with the desired concentration of the EO being tested. The inoculated plates were then incubated at 24°C.

The results were obtained by visually examining and measuring the diameter of the fungal growth inhibition zones surrounding the discs loaded with the various EO volumes after 3 days for *Aspergillus niger*, and 6 days for *Zymoseptoria tritici*. All treatments were performed in triplicate for each EO volume tested.

1.4. Data treatment and analysis

EXCEL 2019, was used for the treatment and analysis of obtained results (average values of inhibition zone diameter, histograms,…).

Results and Discussion

Chapter 02: Results and discussion

2.1. Yields of Essential oils

The yields of essential oils obtained from studied plants, as represented in **Figure 10**, reveal that *Thymus vulgaris* yields have the highest amount, with a value of 1.265 ± 0.025%, follows by *Lavandula stoechas ssp. pedunculata* with a yield of 0.882 ± 0.12%, but *Ruta montana* exhibits the lowest yield with 0.250 ± 0.054 %.

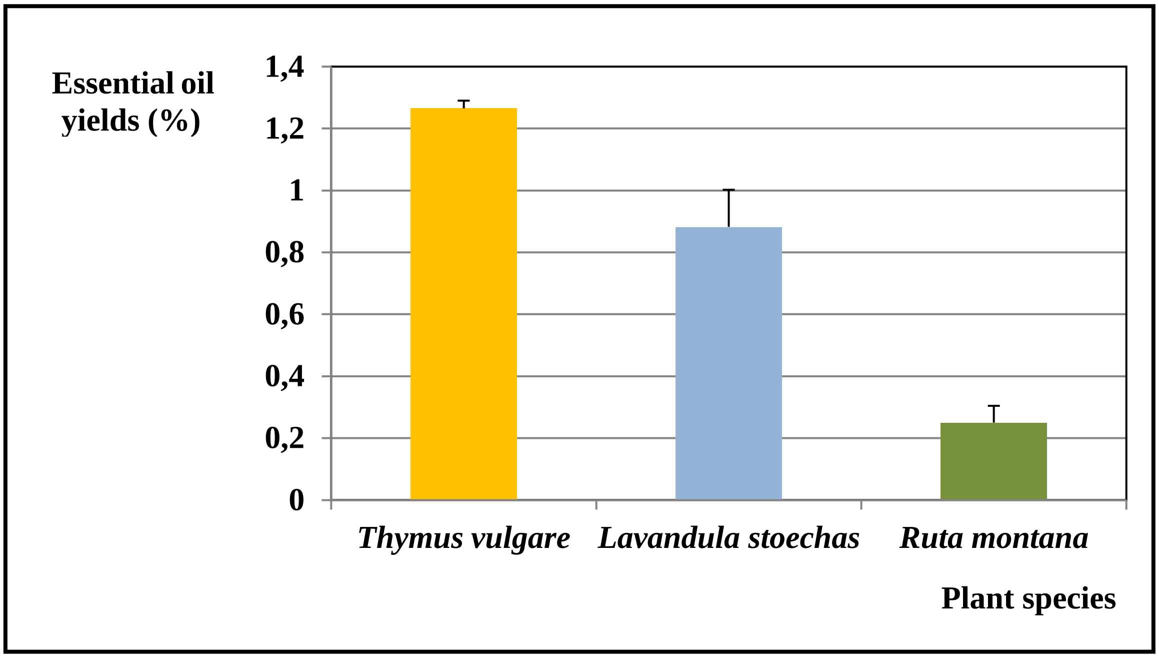

Figure 11: Essential oils yield of the plants used in this study.

When comparing our findings with the existing literature, we observe that:

- **For *Thymus vulgaris*:** The extraction yield of the essential oil obtained in this study, is higher than those obtained by **Imeloune & *al*. (2009)**; using the same method, the authors obtained yields of 1.0% from dried aerial parts of plants collected during the flowering period in eastern Morocco. **Shabnum & Wagay (2011)** have use the same plant, collected at the flowering stage, from northern Italy, recorded a highest yield than what we found using the same method of extraction (Hydrodistillation using clevenger type apparatus for 3 hours) with a value of 1.6%. In other studies, using the same species of *Thymus*, a lower yield, comparing with ours, were registered, by using the oven drying method (0.5%) and the wire basket dryer method (0.6%). Many authors, reveals that the method of extraction can affects the yield obtained by the plants.

Pirbalouti & *al*. (2013) have used *T. daenensis* and *T. vulgaris*, from different regions (100 g of powdered plant material), with hydrodistillation method, using Clevenger type apparatus, has obtained 0.75 ± 1.16 %.

- **For *Lavandula stoechas* ssp. *pedunculata*:** The extraction yield of the essential oil from the dry material is 0.882 ± 0.12% which is considered lowest than that obtained by **Msaada & *al*. (2012)** which used the aerial parts (flowers, leaves and stems) of *L. dentata*, *L. stoechas* and *L. multifida* collected from Grombalia greenhouse (the North-Eastern Tunisia) during the flowering stage, applied the same method of the extraction of the essential oil, they obtained : 1.96 ± 0.22 % for *L. dentata*, 1.62 ± 0.15 % for *L. multifida* and 1.04 ± 0.11 % for *L. stoechas* , on dry weight basis. **Barkat & Laib (2012)**, reported a significant yield of 3.41%, for the same species collected from Constantine, Algeria in June 2010, at flowering stage, using the same method of extraction.

Our results are more important from those obtained by **Zuzarte & *al*. (2013).** The authors have recorded a yield of 0.7 % from the aerial parts of the plant collected from Sardinia Island (Italy).

- **For *Ruta montana*:** The average yield from three consecutive extractions was 0.249 ± 0.054 %, and it is lower than those obtained by **Bejaoui & *al*. (2019)**, who have use the same method from the same plant collected from Bizert (Tunisia) during the flowering stage, they have used the aerial parts of the plant, and obtained a yield of 1.21%. It is also lower than those obtained from the same plant growing in Souk Ahras (Algeria) by **Slougui & *al*. (2023)**, who founds a value of 0.67 %, by using the aerial parts of the plant (stem and leaves) collected at the end of January 2020.

Belhadi (2016) have used the leaves of the same plant, collected from three different regions of Algeria: Batna, Blida and Bejaia, in flowering stage, and with hydrodistillation method, It has recorded a yield of 2,53%, 2,01% and 1,37%, respectively.

2.2. Organoleptic properties of the essential oils

The quality of an essential oil (EO) is determined by various factors, including its aspect (appearance), color, and aroma. These organoleptic properties, which are perceived by the senses, are key indicators of an oil's quality.

Table 02 illustrates the organoleptic characteristics of the essential oil extracted in our study. It likely provides details on the specific appearance, color range, and scent profile that define the quality for the different oils studied.

Table 02: Organoleptic properties of essential oils extracted in this study

Origin of EOs	Color	Smell	Aspect
Ruta montana	Yellowish	Strong smell	Liquid
Lavandula. stoechas ssp. *pedunculata*	Pale yellow	Fresh strong smell	Liquid
Thymus vulgare	Yellow	Fresh strong smell	Liquid

2.3. Antifungal activity results

2.3.1. Potential of antifungal activity of EOs on *Aspergillus niger*

2.3.1.1. Effects of *Thymus vulgaris* EO on the growth of *Aspergillus niger*

For *Thymus vulgaris* essential oil experiments, we have used four different volumes which are 0µL, 2.5µL, 5µL, 10µL and 20µL. We marked the highest average inhibition zone at 20µL of essential oil with a value of 20 ± 0 mm (**Fig. 11**). 2.5µL have recorded the lowest average inhibition zone with 8 ± 0 mm.

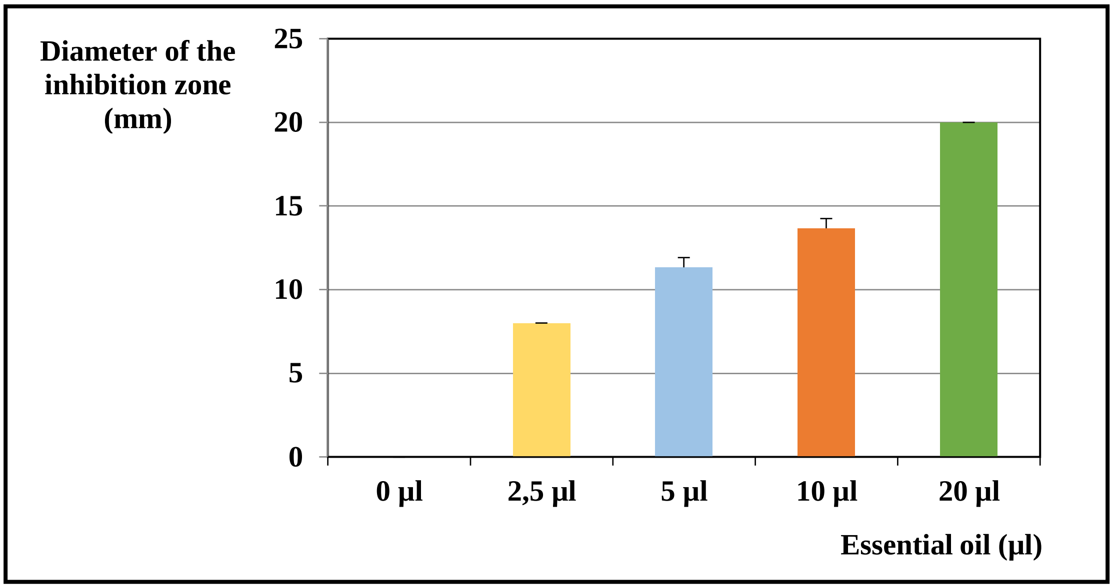

Figure 12: Diameters of the inhibition zones of *Aspergillus niger* versus *Thymus vulgaris* essential oil

Our results can be considered like important compared with those of **Rasooli & Abyaneh (2003).** The authors, have studied inhibition of *Aspergillus parasiticus* growth and its aflatoxin production exposed to the essential oils extracted from two varieties of Thyme i.e. *Thymus eriocalyx* and *Thymus x-porlock*. The disc diffusion method was used to evaluate the zone of fungal growth inhibition at various concentrations of the oils. The results obtained have shown that, fungal growth inhibition was noted for both plant essential oils. The oils from *T. eriocalyx* severely inhibited fungal growth at the first three dilutions of 1, 1/2 and 1/4. The same observation was noted when the fungus was exposed to undiluted oil of *T. x-porlock*. Diameters of 13, 11 and 9 mm fungal growth inhibition zones were observed at 1/8 and 1/16 dilutions of *T. eriocalyx* oil respectively. The fungus was resistant to dilution 1/32 of this oil.

Abdi-Moghadam & *al*. (2023), have used various concentrations of *Thymus (Thymus eriocalyx* and *Thymus x-porlock)* essential oil and get an average inhibition zone diameter, between 8 and 25 mm.

Viuda-Martos & *al*. (2006), reported that in the case of *A. niger* the thyme essential oil reduced fungal growth at 2µL/18 mL culture medium, and the total inhibition was only attached when 8 µL was used. And in the case of *A. flavus*, thyme EO reduced mycelial growth at 2,4 and 6 µL. Inhibition was total at 8 µL. These values was higher than those obtained with *A. niger*, that means that *A. flavus* was more sensitive to thyme essential oil than *A.niger*.

The potential of the antifungal activity of *Thymus* sp. essential oils, will be attributed at its composition and its richness in four important components: thymol, b-phellandrene, cis-sabinene hydroxide, b-pinene, which had a huge impact on fungi. **Bouddine & *al*. (2012)**, have evaluate the antimould activity of oregano, thyme, rosemary and clove essential oils and some of their main constituents: eugenol, carvacrol and thymol against *Aspergillus niger*. This antifungal activity was assessed using broth dilution, disc diffusion and micro atmosphere methods. In both agar diffusion and micro atmosphere tests, all the investigated agents showed no inhibitory effect on *Aspergillus niger* growth at concentrations lower than 10% (v/v). However, broth dilution test showed the highest sensitivity. The anti-*Aspergillus* effect of oregano and thyme oils was more potent than that of clove and rosemary oils. Concerning the phenolic compounds, thymol and carvacrol proved to have better anti-*Aspergillus* effect than eugenol.

The results obtained by the same authors, for the antifungal effect of the studied EOs and some of their major components by micro atmosphere test reveals that, at concentrations of 1, 0.2 and 0.1%, no inhibition zone was obtained for all the tested agents. However, these agents at 10% (v/v) show an antifungal activity against *A. niger* with inhibition zones from 40 to ≥ 85 mm. Concerning the EOs, the largest inhibition zone was observed with oregano (≥ 85 mm). Thyme and clove oils show moderate effect with inhibition zones of 70 and 40 mm, respectively. No activity was noticed with rosemary oil. Among the tested phenolic compounds at 10%, thymol gave a larger inhibition zone (≥ 85 mm) than carvacrol (56 mm) and eugenol (49 mm).

2.3.1.2. Effects of *Lavandula stoechas* EO on the growth of *Aspergillus niger*

Figure 12 presents the effects of the EO of *L. stoechas* ssp. *pedunculata* on the growth of *Aspergillus niger*. We can see that all of the volumes used in this study, can inhibit the growth of the fungus. The highest average inhibition zone was at 40 µL with a value of 15.666 ±0.577 mm. The lowest average inhibition zone was at 5µL with 11 ± 1mm.

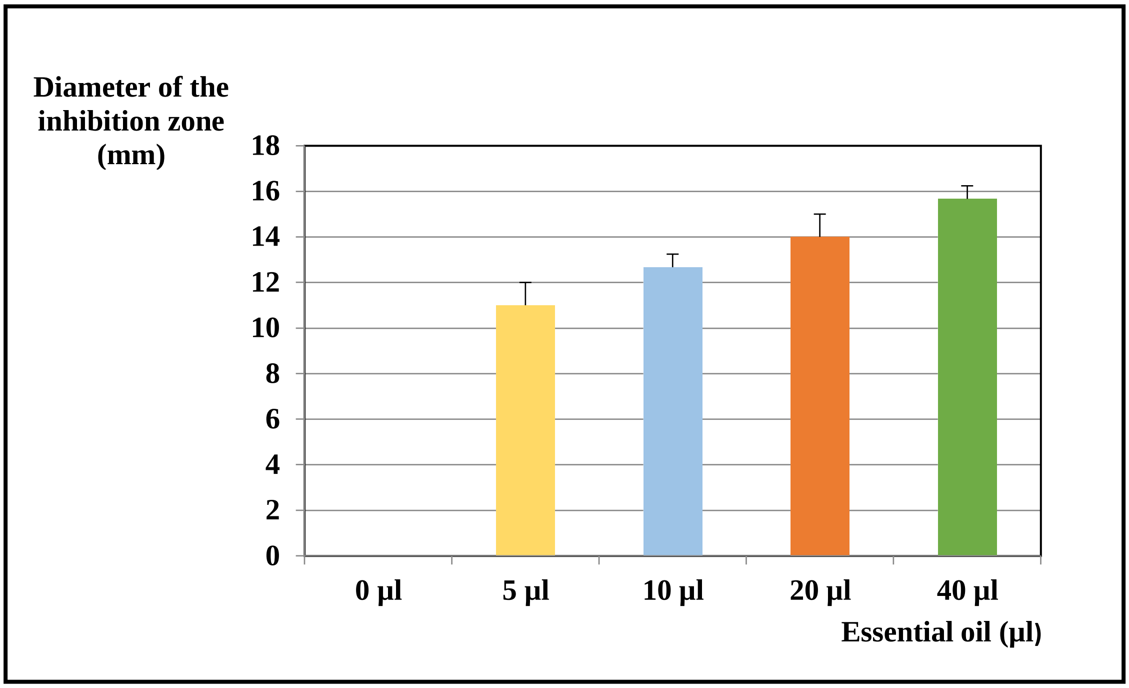

Figure13: Diameters of the inhibition zones of *Aspergillus niger* versus *Lavandula stoechas* essential oil

Uniyal & *al.* (2012), have used the aerial parts of sixteen aromatic plants including *Lavandula angustifolia* which were collected from Dehradun district in northern India, the authors have noticed that 20 µL of *L. angustifolia* essential oil showed a significant values of inhibition zone (20 mm against *A. niger*, 15 mm against *A. fumigatus*).

Based on the study of **Rostaminejad & *al.* (2022)**, *L. stoechas* EOs, contain different compounds which are Fenchone 50.29-55079%, D-fenchone 29.28%, α-pinene 23.18%, Camphor 14.02-18.18%, 1,8-Cineole 8.03%, Carvacrol 7.8%, ….

Cisarovà & *al.* (2016), have evaluate the antifungal activity of lemon (*Citrus lemon* L.), eucalyptus (*Eucalyptus globulus* LABILL.), thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.) sage (*Salvia officinalis* L.) and lavender (*Lavandula angustifolia* MILLER.) EOs against *Aspergillus niger* and *Aspergillus tubingensis* isolated from grapes and their ability to affect the growth. It was tested by using the vapor contact with them. The effect of the EO volatile phase was confirmed to inhibit growth of *A. niger* and *A. tubingensis*. The most effective tested EOs were oregano and thyme oils, which totally inhibited growth of tested isolates for all days of incubation at 0.625 µL.cm-[3] (in air). Lavender EO was less active aginst tested strains. The results showed that the tested EOs had antifungal activity, except lemon and eucalyptus. Sage EO was the only one which decelerated the radial growth of colony of both tested strains after all days of cultivation in comparison with a control sets.

2.3.1.3. Effects of *Ruta montana* EO on the growth of *Aspergillus niger*

The results obtained in this study are presented in **figure 13**. All of the volumes tested of *R. montana* essential oil have inhibit the growth of the fungus (*A. niger*), 40µL was the most effective volume compared to the others with a value of 12.666 ± 0.577 mm. While 5µL gives the lowest effect with 8 ± 1mm of inhibition zone diameter.

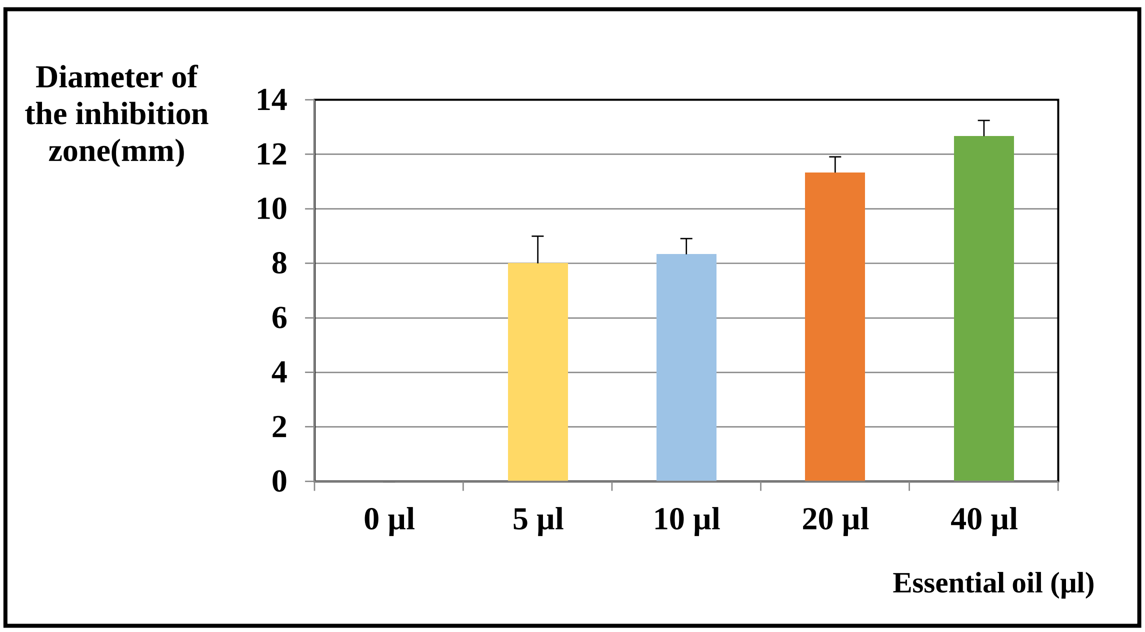

Figure 14: Diameters of the inhibition zones of *Aspergillus niger* versus *Ruta montana* essential oil

Abdi and Tirouche (2022) obtained for this fungus an inhibition zone of 9 mm for all of the volumes tested (5, 10, 20 and 40 µL), using the aerial parts (leaves, flowers and twigs) of *Ruta graveolens* and get a yield of 0.750 ± 0.057 %, so our results considered like important compared to their results. They have also obtained an inhibition of the growth of *Botrytis cinerea* but only at 40µL where they have recorded an inhibition zone exceeding 1 cm.

Reddy and Al-Rajab (2009), obtained an inhibition zone of 25.60 ± 0.02mm of *Ruta grievances L*. essential oil against *Aspergillus flavus* and an inhibition zone of 14.30 ± 0.06 mm against *Aspergillus fumigates* at 10 µL/disc utilizing the aerial parts of the plant material during the flowering stage from Rayachoty, Rayalaseema region, in northern India.

Drioiche & *al*. (2020) noticed that, essential oils extracted by hydrodistillation of *Ruta montana* L., collected in the Boulemane region (Morocco); yielded 1.60 ± 0.08 % and GC/MS analysishave shown thirty-two components with the dominance of 2-undecanone (82.62 %), 2-undecanol (2.87 %) and 2-undecanol acetate (2.13 %). Phytochemical screening of aerial parts of *Ruta montana L*. revealed the presence of alkaloids, mucilages, triterpenes, sterols, flavonoids, leucoanthocyanes, gallic and catechin tannins, as well as combined anthracene derivatives. Phenolic compounds of *Ruta montana* were extracted by Soxhlet and by maceration with yields of 17.92 ± 0.90 % and 11.03± 0.55 % respectively.

Nahar & *al*. (2021) reveals that the genus *Ruta* is rich in essential oils, which predominantly contain aliphatic ketones, e.g., 2-undecanone and 2-nonanone, but lack any significant amounts of terpenes. Three *Ruta* species, *Ruta chalepensis* L., *Ruta graveolens* L., and *Ruta montana* L., have been extensively studied for the composition of their essential oils and several bioactivities, revealing their potential medicinal and agrochemical applications. The essential oil of *Ruta montana L*. from Algeria (Blida, Bouira, Boumerdes, Djelfa, Msila, Tipaza and Tizi ouzou) contains 2-Undecanone (27.2-81.7%), 2-nonanone (1.9-39.5%), and 2-nonanyl acetate (trace-24.8%), and it exhibited an inhibition zone of 12 mm in diameter against *Aspergillus niger*.

2.3.1.4.* Effects of the combination *Ruta montana* and *Lavandula stoechas ssp pedunculata* EOs on the growth of *Aspergillus niger

According to the results obtained by this study (**Fig. 14**), all the tested volumes of the mixture of *R. montana* and *L. stoechas* have inhibited the growth of *Apergillus niger*. At 40µL we noted an important effect on the growth of the fungus with 17.666 ± 0.577 mm of inhibition zone diameter. Previously we marked that *L. stoechas* essential oil alone gives also, an important result against *A. niger* (15.666 ±0.577mm at 40 µl, 8 ±1mm at 5µL), and *R. montana* essential oil alone, gives a significant result against this fungus (12.666±0.577mm at 40µL and 11 ± 1mm at 5µL). So, we could assert that the combination between these two oils gives a significant and important antifungal activity more than the use of these two EOs separated.

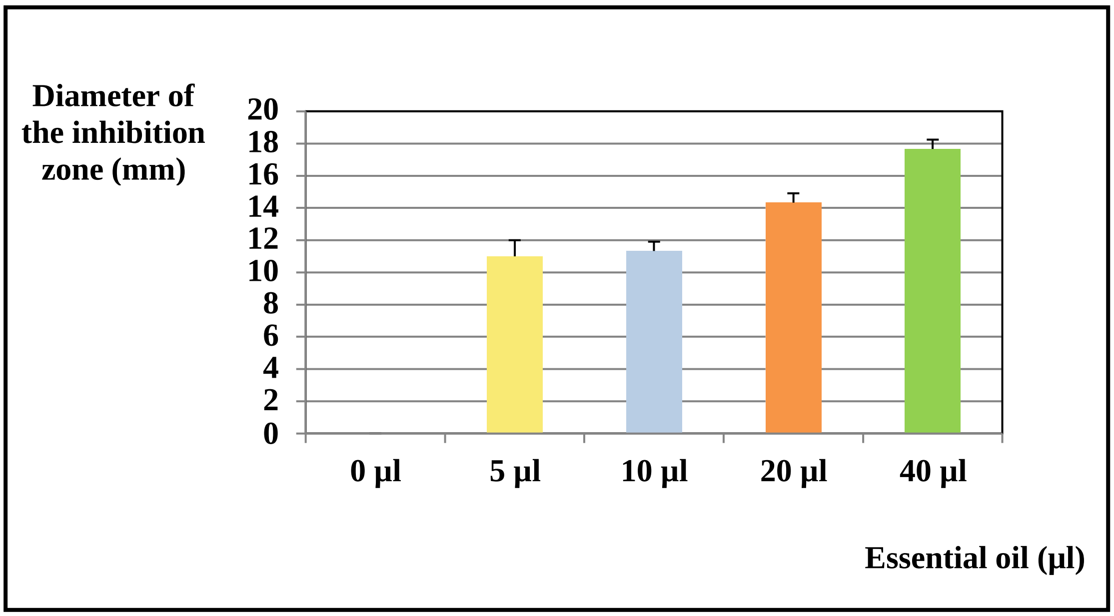

Figure 15: Diameters of the inhibition zones of *Aspergillus niger* versus *Ruta montana* + *Lavandula stoechas ssp pedunculata* essential oils

Özcan & *al*. (2018), reported that the volatile fraction of *L. stoechas* oil stands out from that the other *Lavandula* species in the Mediterranean region. The oil of *L. stoechas* primarily consist of monoterpenoid alcohols such as linalool, ɑ-terpineol, and terpinene-4-ol. In contrast, the oil of *L. stoechas* ssp. *atlantica* is characterized by a significant presence of camphor (39%) and fenchone (9%), whereas *L. stoechas* ssp. *stoechas* oil is dominated by fenchone (30%) and camphor (18%).

2.3.2. **Potential of antifungal activity of EOs on *Zymoseptoria tritici***

2.3.2.1. **Effects of *Thymus vulgaris* EO on the growth of *Zymoseptoria tritici***

Figure 15 presents the effects of the EO of *Thymus vulgaris* on the growth of *Zymoseptoria tritici*. The volume 20µL was the most effective one against *Zymoseptoria tritici* with 20 ±1 mm of the inhibition zone diameter. At 2.5 µL, the inhibition zone diameter was also important, with a value of 8 ±1 mm.

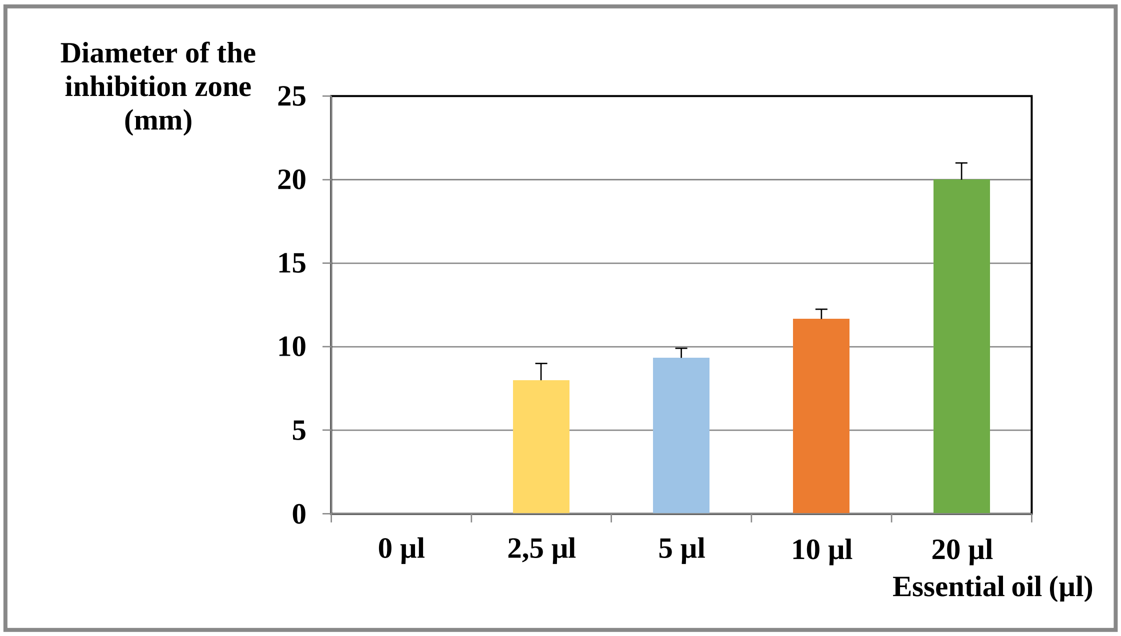

Figure 16: Diameters of the inhibition zones of *Zymoseptoria tritici* versus *Thymus vulgaris* essential oil

According to the study of **Mota & *al.* (2012)**, *T. vulgaris* EO contains 10 chemical compounds, the most abundant element was thymol 46.6%. They have tested many Eos and have confirmed that *Thymus* EO is one of the essential oils that shows a strong and wide spectrum of antifungal action and displayed the best activity. So, a large number of essential oils, especially those of some species of *Thymus* and their phenolic components have been investigated for their antimicrobial properties against certain bacteria, protozoans and fungi. The essential oil of *T. vulgaris* shows one of the best antifungal profiles in fungal susceptibility tests using the disk diffusion assay in solid medium.

Anžlovar & *al*. (2014), who planted the seedlings of common thyme (*Thymus vulgaris*) under a greenhouse conditions, and transplanted to the experimental field of the Biotechnical Faculty in Slovenia, and tested the oil on different bacteria and fungi, informed that the antifungal activity of *Thymus* EO was weaker than the antibacterial activities, as fungal growth was inhibited only at the highest tested fraction, at 10% of essential oil. The mean diameter of the growth inhibition zone of these fungi that grew in the presence of 10% essential oil were 4.1mm for *Fusarium sp.*, but against bacteria *S. aureus* for example it gives 1.9 ± 0.3mm diameter at 1% of the EO and 7.0 ± 1.3 mm diameter at 10% of EO.

Comparing with our results thyme essential oil was more effective against *Zymoseptoria tritici* at the high volumes (20 ± 1mm at 20 µL) and at the lower volumes (8 ±1mm at 2.5µL). **Dewitte & *al*. (2018)**, noted that thyme essential oil showed almost 100 % growth inhibition of the wheat pathogens including *Zymoseptoria tritici* at a concentration of 1µLml^{-1}.

2.3.2.2. Effects of *Lavandula stoechas ssp pedunculata* EO on the growth of *Zymoseptoria tritici*

L. stoechas essential oil shows an important effect on the growth of *Zymoseptoria tritici* at the volume of 40µL with a value of 12.333 ± 0.577mm (**Fig. 16**). At the lowest used volume (5µL), the inhibition zone diameter was 8.333 ± 0.577mm.

Boudjehem (2019) who have used diluted EO of the same plant (*Lavandula stoechas*) collected from Guelma region (Mahouna), have found that, no inhibitory effect on the growth of *Zymoseptoria tritici* was observed at a concentration of 25 %. However, at concentration of 50 % and 75 % and 100% (pure oil), a diameter of the inhibition zone was observed, and it is more important at 100 %.

Lamoudi & *al*. (2023), who used the flowers of *L. stoechas* collected from the north Algeria, found fifty compounds, and the major ones were fenchone (29.42 %), camphor (13.41%), lavandulyl acetate (13.0 %) and cineole (8.87%) accounting for more than 60% of the total oil in the analyzed samples. The authors noted that the EO of *L. stoechas* was very active against *Candida albicans*.

Moderately active against *E. coli*, *Bacillus subtilis*, *Pseudomonas aeuginosa*, with an inhibition zone diameter varying from 12.0 to 32.0 mm.

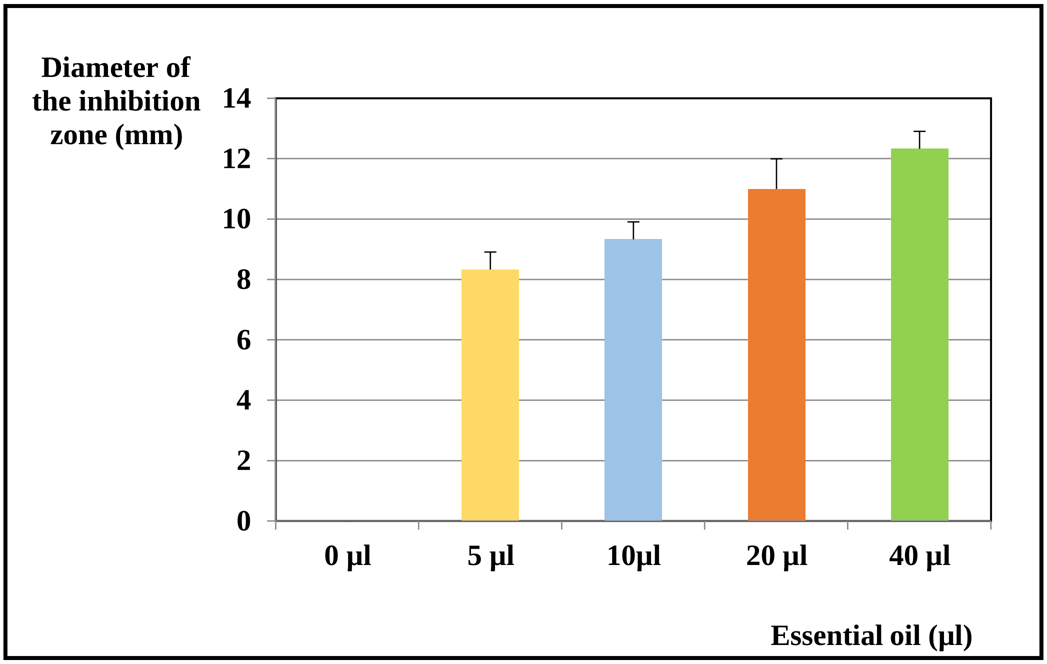

Figure 17: Diameters of the inhibition zones of *Zymoseptoria tritici* versus *Lavandula stoechas ssp pedunculata* EO

2.3.2.3. Effects of *Ruta montana* EO on the growth of *Zymoseptoria tritici*

Figure 17 show that *Ruta montana* EO have moderate effect on the growth of *Zymoseptoria tritici*. Comparing the average inhibition zone values for *Zymoseptoria tritici* we can observe that, the volume of 40µL of the essential oil has the highest average inhibition zone with a value of 14 ± 1mm, and at the lowest used volume, the inhibition zone is important (9.666 ± 0.577 mm).

Comparing with our results of *R. montana* EO against *A. niger* we can see that this oil is more active against *Z. tritici* than *A. niger* because we have found a lower average inhibition zone against *A. niger* at the highest volumes (12.666±0.577mm at 40µL and 8 ±1mm at 5µL) than that against *Z. tritici*.

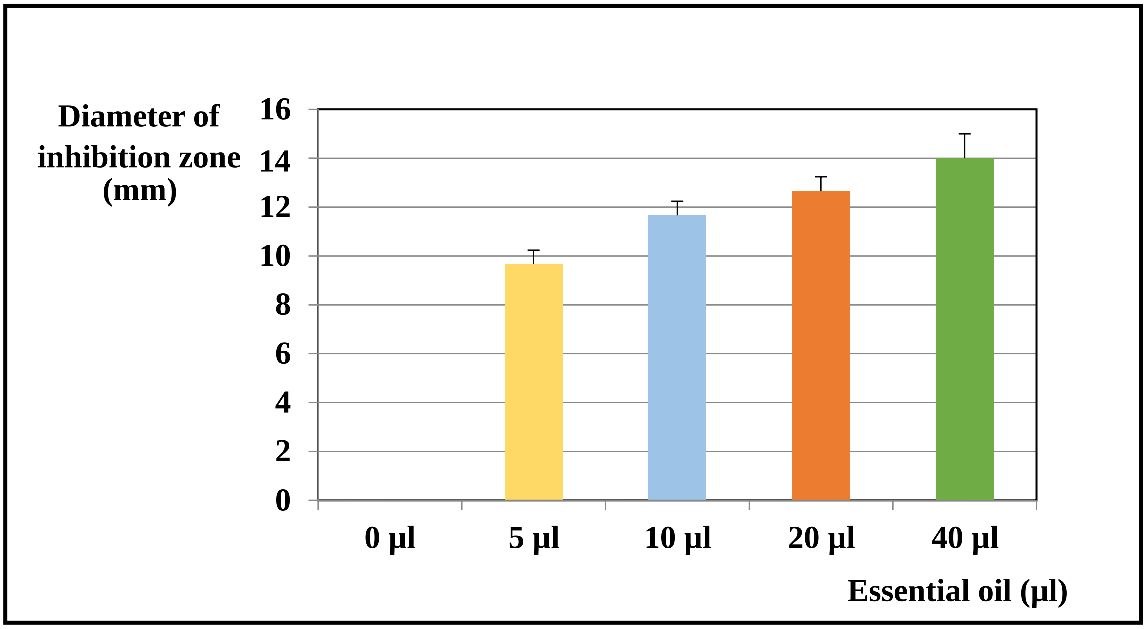

Figure 18: Diameters of the inhibition zones of *Zymoseptoria tritici* versus *Ruta montana* essential oil

2.3.2.4.* Effects of the combination *Ruta montana* and *Lavandula stoechas ssp pedunculata* EOs on the growth of *Zymoseptoria tritici

Figure 18 presents the effects of the EO of *Ruta montana* + *Lavandula stoechas* ssp *pedunculata* on the growth of *Zymoseptoria tritici.* The average inhibition zone values for *Zymosetoria tritici* reveal that the mixture of the two essential oils (*Ruta Montana* EO + *L. stoechas* EO) at 40µL exhibits the highest average inhibition zone with a value of 21.333 ± 0.577mm, while at 5µL the mixture of the two EOs has a lowest average inhibition zone with 10 ± 0 mm.

Comparing with the previous results the EO of *L. stoechas* alone gives 12.333±0.577mm of the inhibition zone at 40µL and 8.333±0.577mm at 5µL, and in the other hand *R. montana* oil gives 14 ± 1mm at 40µL and 9±0.577mm at 5µL. So, we can see clearly that the combination of *L. stoechas* and *R. montana* EOs has a huge antifungal activity against *Z. tritici* in high volumes which gives an average inhibition zone with 21.333±0.577mm at 40µL and 10±0mm at 5µL which is considered like very important compared with the result obtained with the two oils separated.

Also results obtained with the mixture of these two oils against *A. niger*, shows an inhibition zone of 17.666±0.577mm at 40 µL and 11±1mm at 5µL which is lower than the value obtained against *Z. tritici*. Based on these results, we confirm that the combination between these two oils is more active against *Z. tritici* than *A. niger*.

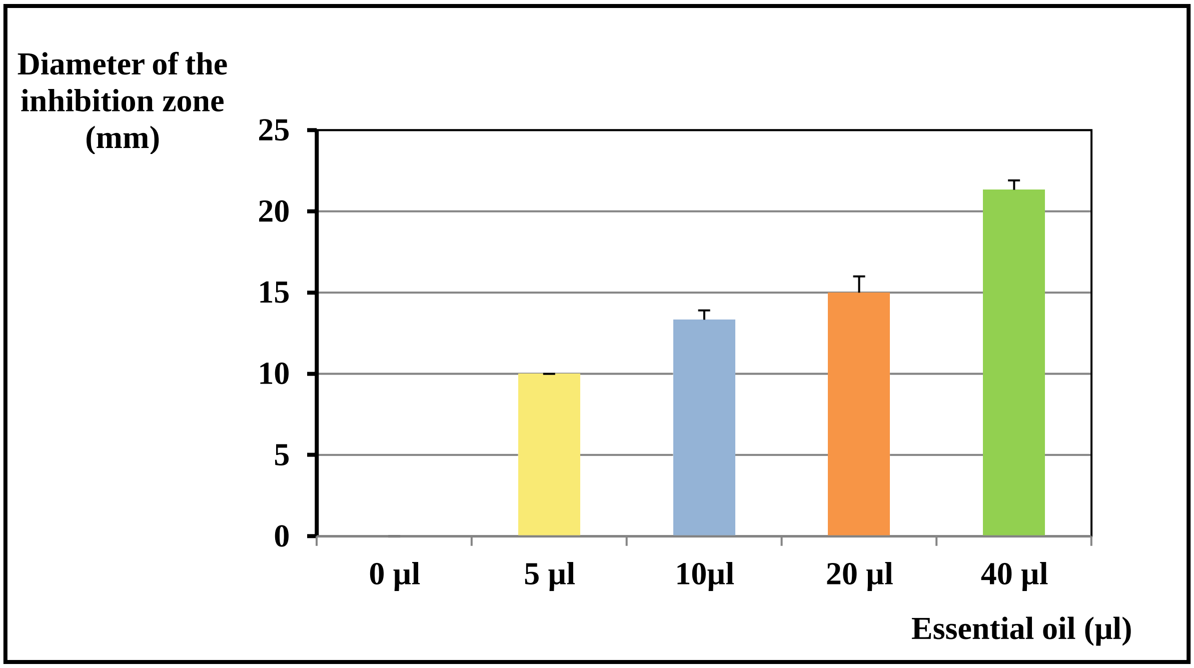

Figure 19: Diameters of the inhibition zones of *Zymoseptoria tritici* versus *Ruta montana* + *Lavandula stoechas ssp pedunculata* essential oils

Conclusion

Conclusion

Biopesticides play a crucial role in sustainable agriculture, providing an alternative to conventional chemical pesticides. These natural substances are derived from living organisms such as plants, bacteria, fungi, or even some minerals. They provide effective pest control while minimizing the negative impact on the environment, human health and non-target organisms.

A major advantage of biopesticides is their ability to target specific pests while leaving beneficial insects unharmed. For example, *Bacillus thuringiensis* (Bt) is a bacteria commonly used as a biopesticide. It produces proteins that are toxic to certain insect pests, such as caterpillars, while they are safe for other organisms.

Reducing environmental impact unlike chemical pesticides, biocides are generally less persistent in the environment. They decompose more quickly, reducing the risk of them accumulating in soil, water and food. This characteristic contributes to the overall sustainability of agricultural practices.

Phytobiopesticides are derived from plant extracts and contain natural compounds that have pesticide properties. Naturally sourced biocides contain one or more compounds originating from nature. Many of them are vegetable oils and plant extracts. Plant extracts and oils can come from different parts of the plant, such as the fruits, bark, leaves, or seeds. They are primarily used against insect pests, but some can also control plant diseases.

In this study we have determinate the yield of essential oils extracted from three plants, that grow naturally in Guelma region of Algeria (*Thymus vulgaris*, *Ruta montana* and *Lavendula stoekas*), and evaluate their antifungal activity against two common fungal pathogens (*Aspergillus niger*, *Zymoseptoria tritici*), responsible for black 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 yields of essential oils obtained from studied plants, reveal that *Thymus vulgaris* yields the highest amount, with a value of 1.265 ± 0.025%, follows by *Lavandula stoechas* ssp. *p*edunculata with a yield of 0.882 ± 0.12%, but *Ruta montana* exhibits the lowest yield with 0.250 ± 0.054 %.

- For the antifungal activity, our results reveals that :

- All EO tested volumes were effective against the studierd pathogens.
- Inhibition of fungi growth increases with the volume of EO.
- *Aspergillus niger,* was more sensitive to *Thymus vulgaris* EO, moderately sensitive to *Lavandula stoechas* ssp. *pedunculata* EO, and less sensitive to *Ruta montana* EO.
- *Zymoseptoria tritici* was more sensitive to *Thymus vulgaris* EO, moderately sensitive to *Ruta montana* EO, and less sensitive to *Lavandula stoechas* ssp. *pedunculata* EO.
- Combination of *Lavandula stoechas* ssp. *pedunculata* EO and *Ruta montana* EO has shown, more antifungal activity against *Zymoseptoria tritici* and *Aspergillus niger* and gives an average inhibition zone which is considered like very important compared with the results obtained with the two oils separated, and the combination of these two oils is more active against *Z.tritici* than *A.niger*.

These results suggest that *Thymus vulgaris, Ruta montana* and *Lavandula stoechas* ssp. *pedunculata* EO, could be used economically as biopesticides to control the studied pathogens, and probably, as valuable bioproduct with new functional properties in the food and pharmaceutical industries.

In perspective, the EO and extracts of these plants require more research on their chemical composition and their application. Others studies on the antimicrobial properties of those plants or other plant species of Algeria, will provide more informations of their biopesticide potential and their applications in agriculture, as alternative natural products to synthetic pesticides, in order to protecting the environment for agricultural crops, and reducing the risk of developing resistance to pests.

Abstracts

Abstract

This study investigates the properties and antifungal potential of essential oils extracted from *Thymus vulgaris*, *Lavandula stoechas ssp. pedunculata*, and *Ruta montana*, which grow naturally in the Guelma region of Algeria. The plants were tested against *Aspergillus niger* 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 *Thymus vulgaris*, followed by *Lavandula stoechas* and then *Ruta montana*. Antifungal activity is evaluated using the disc diffusion method for oils using 4 treatments (*Thymus vulgaris* EO, *Lavandula stoechas* EO, *Ruta montana EO*, and a mixture of *Lavandula stoechas* EO *and Ruta montana* EO v/v), and 4 volumes of pure EO for each treatment/pathogen. The study reveals satisfactory antifungal activity against the studied pathogens at the tested volumes of essential oils from all plant species. These results contribute to understanding the antifungal potential of these essential oils, highlighting their potential as natural alternatives to control plant pathogens and reduce crop losses while protecting the environment.

Key words: *Thymus vulgaris*, *Lavandula stoechas*, *Ruta montana*, essential oils, antifungal activity, *Aspergillus niger*, *Zymoseptoria tritici*.

Résumé

Cette étude vise à déterminer les propriétés et le potentiel antifongique des huiles essentielles extraites de *Thymus vulgaris*, *Lavandula stoechas ssp. pedunculata* et *Ruta montana*, qui poussent naturellement dans la région de Guelma en Algérie. Les plantes ont été testées contre *Aspergillus niger* 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 HE est obtenu àpartir de *Thymus vulgaris,* suivi de *Lavandula stoechas* et enfin de *Ruta montana*. L'activité antifongique est évaluée par la méthode de diffusion directe des HE à travers des disques de papier wattman, en utilisant 4 traitements (HE de *Thymus vulgaris,* HE de *Lavandula stoechas,* HE de *Rutamontana*, et un mélange d'HE de *Lavandula stoechas* et d'HE de *Ruta montana* v/v), et 4 volumes d'HE pure pour chaque traitement/agent pathogène. L'étude révèle une activité antifongique satisfaisante contre les agents pathogènes étudiés aux volumes testés d'huiles essentielles de toutes lesespèces végétales étudiées. Ces résultats permettent de comprendre le potentiel antifongique de ces huiles essentielles, mettant en évidence leur potentiel en tant qu'alternatives naturelles pour contrôlerles pathogènes des plantes et réduire les pertes des récoltes tout en protégeant l'environnement.

Mots clés : *Thymus vulgaris*, *Lavandula stoechas*, *Ruta montana*, huiles essentielles, activité antifongique, *Aspergillus niger*, *Zymoseptoria tritici*.

ملخص

تناولت هذه الدراسة تحديد الخصائص والإمكانات المضادة للفطريات للزيوت العطرية المستخرجة من *Thymus vulgaris*، *Lavandula stoechas ssp. pedunculata* و *Ruta montana* التي تنمو بشكل طبيعي في منطقة قالمة بالجزائر. تم اختبار النباتات ضد *Aspergillus niger* و *Zymoseptoria tritici*. تم استخدام تقنية التقطير المائي لاستخلاص الزيوت الأساسية ، باستخدام جهاز من نوع Clevenger، تم الحصول على أعلى إنتاج من الزيوت الأساسية من *Thymus vulgaris* ، يليه *Lavandula stoechas* ثم *Ruta montana*. تم تقييم النشاط المضاد للفطريات باستخدام طريقة الانتشار المباشر من خلال أقراص لورق واتتمان (Wattman) باستخدام 4 علاجات (EO *Thymus vulgaris*، و EO *Lavandula stoechas*، و EO *Ruta montana*، ومزيج منن EO *Lavandula stoechas* و v/v EO *Ruta montana*)، و4 احجام من الزيت ا الأساسي الخالص لـ كل علاج/مسبب المرض. كشفت الدراسة عن نشاط مضاد للفطريات مرضٍ ضد مسببات الأمراض المدروسة في الكميات المختبرة من الزيوت ا الأساسية من جميع أنواع النباتات. وتساهم هذه النتائج في فهم الإمكانات المضادة للفطريات لهذه الزيوت الأساسية، وتسليط الضوء على امكانية استعمالها كبدائل طبيعية للمبيدات للسيطرة على مسببات الأمراض النباتية وتقليل خسائر المحاصيل مع حماية البيئة.

الكلمات المفتاحية: *Tymus vulgaris*، *Ruta montana*، *Lavandula stoechas*، الزيوت ا الأساسية، النشاط المضاد للفطريات، *Zymoseptoria tritici*، *Aspergillus niger*.

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