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Identification of essential oils of *Mentha rotundifolia* from north-east Jijel region and their antifungal potential against *Botrytis cinerea*

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Abstract: The objective of the present research is to determine the main essential oils of *Mentha rotundifolia* and to clarify the antifungal effect of these oils against *Botrytis cinerea* isolated from strawberries. The essential oils were obtained from hydrodistillation of fresh leaves, and the composition was determined using gas chromatography/mass spectrometry (GC/MS). The results show that the essential oils yield is in the order of 1.37%, and the main components were identified as pulegone 39.21%, ß-cubebene 11.72%, caryophyllene 9.18%, D-limonene 4.7%, ß-ocimene 2.39%, myrcene 2.21%, myrtenol 2.1%, ß-farnesene 2.03%, respectively. The essential oils of *M. rotundifolia* have been shown to be effective against *B. cinerea* in terms of antifungal activity, recording significant statistical results ($P < 0.0001$). *M. rotundifolia* essential oils have an inhibitory effect on mycelian growth, as evidenced by the reduction or inhibition of mycelian growth with increased oil concentrations of (60%) and (100%), with inhibition rates ranging from 60.83% to 48.07%, respectively. The activities of the essential oils indicated that *B. cinerea* was a sensitive strain tested to the oils of *M. rotundifolia*. According to the findings, the plant under consideration is promising as a source of natural fungicides and lends itself well to research in the field of fungi control using biological alternatives.

Keywords: pulegone; hydrodistillation; chromatography; aromatogram; fungi

Lamiaceae species are found all over the world, with varying heights and habitats and greater abundance in the Mediterranean region, including the *Mentha* genus, which contains some of the world's most commonly cultivated spice plants (Kallunki 1994). In the Algerian flora, this genus is represented by five major species: *M. rotundifolia*, *M. longifolia*, *M. spicata*, *M. aquatica*, and *M. pulegium* (Quezel, Santa 1962). *Mentha rotundifolia* L. is an aromatic plant widely distributed in the north of Algeria; this family species has a wide range of morphological characteristics and can be herbs, herbaceous plants. The *M. rotundifolia* is a perennial plant that is frequently found at the edge of paths, in ditches or other moist places. The *Mentha* lives for a long time, generally more than two years, producing several blooms and resisting the rigours of the bad season, whether it is winter frost or the dryness of heatwave periods. The country's geographical location and climatic diversity have allowed for the development of a very rich and highly diverse group of aromatic

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and medicinal plants, with over 4 000 species that have been used to treat a variety of diseases since ancient times. Traditional Algerian medicines use decoction and infusion of the rounded-mint leaves to treat a variety of diseases such as hypertension, diabetes, and digestive and genitourinary system disorders (Brahmi et al. 2016). *Mentha rotundifolia* is a hybrid between *M. longifolia* and *M. suaveolens*, whose essential oil has undergone numerous studies and has been classified into various chemotypes (Derwich et al. 2009). The majority of *Mentha* species are perennial and possess essential oils. They are commonly grown as industrial crops to produce essential oils. The *Mentha* genus has been known to be rich in secondary metabolites such as: flavonoids, coumarins, etc.

Fifteen *Mentha* species are scattered across Algeria, and *Mentha* species are some of the most popular food and medicinal herbs in this country. According to Quezel and Santa (1962), the Algerian flora contains five substantial species: *Mentha aquatica* L., *Mentha longifolia* (L.) L., *Mentha pulegium* L., *Mentha rotundifolia* (L.) Huds., and *Mentha spicata* L. However, the last three species are the most exploited. They are commonly used for seasoning food and as herbal remedies to treat many diseases.

M. rotundifolia grows wildly in Algeria, is locally known as "timija" (Brahmi et al. 2017), and used mostly as a condiment but also for medicinal properties. According to Khadraoui et al. (2014), it is known as well as "megne essif" and "timarssat". The species has numerous nouns depending on the region or the country of its distribution, which is the case for *M. rotundifolia*. Its French nouns are menthe à feuilles rondes, menthe du Nil, menthe sauvage (Benazzouz, Hamdane 2012). Meanwhile, Umemoto (1998) affirms that its nouns in the English linguistic are apple-scented mint, false apple-mint.

In Algeria, spontaneous mints are commonly used for both food and phytotherapeutic in coastal and interior regions like Jijel, because of the diverse applications of essential oils of *Mentha* species account for the intense interest in research. However, in the case of our study, *Mentha* is considered a food according to some regions. Where Mentha leaves are dried and ground, mixed with barley flour, and prepared into barley couscous. We then cook and consume them to cure digestive system problems.

In addition, it should be noted that in certain places, especially the Jijel area, fresh *Mentha* leaves are placed in wells and can be added to drinking water to prevent the growth of microbes due to their antibacterial properties. Furthermore, *Mentha* plants are applied to the exterior of water barrels to preserve the coldness of the water. Moreover, mint oils have potent antibacterial properties against Gram-positive and Gram-negative bacteria. Their antimicrobial activity is primarily determined by their chemical composition, specifically the nature of their main volatile compounds. The main group is made up of terpenes and terpenoids, while the other is made up of aromatic and aliphatic constituents, all of which have a low molecular weight (Bakkali et al. 2008). A terpene containing oxygen is called a terpenoid. They serve a variety of purposes. Some terpenes are effective treatments for cancer (Ebada et al. 2010), malaria (Parshikov et al. 2012), and heart disease (Liebgott et al. 2000).

The biological applications of this species are primarily related to its essential oils, which have various activities such as antioxidant (Benabdallah et al. 2018), antibacterial, and insecticidal (Kharoubi et al. 2020); however, they have been little described in the literature. Despite its importance as a medicinal plant, *M. rotundifolia* has limited industrial potential in Algeria. Only a few studies have been conducted on the biological activities of Algerian *M. rotundifolia*, specifically the antibacterial and antifungal effects (Boukhebti et al. 2011). Yet, this research helps to determine the effectiveness of *M. rotundifolia* essential oils against phytopathogenic agents that infect strawberry crops, which is one of the most economically important crops that is consumed worldwide, in order to isolate the main fungi that cause severe diseases.

Botrytis is a cosmopolitan genus with 22 recognised species and one hybrid (Staats et al. 2005), all of which are plant pathogens. According to the previous author, *Botrytis cinerea* is the only species in the genus with a broad host range, whereas all other *Botrytis* species are thought to be specialised on a single plant species. *B. cinerea* has no discernible host preference and can infect over 1 000 plant species. The pathogen is found all over the world and causes disease in a variety of fruit, flower, and leafy vegetable crops (Elad et al. 2016).

Botrytis cinerea is a typical necrotrophic fungus; it kills host plant cells before colonising the dead tissue (Amselem et al. 2011). The minimum temperature for growth is 0 °C, the optimal temperature

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is 20 °C, and the maximum temperature is 30 °C. As a result, the pathogen thrives during the cold storage of fruits and vegetables (Romanazzi, Feliziani 2014). Strawberry infections are often caused by *B. cinerea* postharvest decay, which is one of the most common fungal pathogens. *Botrytis cinerea* (teleomorph: Botryotinia fuckeliana) is a necrotrophic airborne plant pathogen that attacks over 200 crop hosts worldwide (Williamson et al. 2007) and the causal agent of the grey mould disease. It reproduces primarily through asexual conidia or spores, which are easily dispersed by wind, water, or any physical activity. It affects the vegetable and fruit crops, as well as many shrubs, trees, flowers, and weeds (Amselem et al. 2011). This Ascomycete can infect a wide variety of plants at any stage of development and is found on all continents. *Botrytis cinerea* has the potential to cause significant economic losses in a wide range of crops and harvested commodities, including vegetables (lettuce, tomato), fruits (berries, kiwifruit), and ornamentals (rose). As a result, this current infection causes significant economic crop loss in preharvest stages such as growing seasons, harvesting time, and handling processes, as well as postharvest stages such as transportation and storage conditions. Furthermore, fungi are the most economically important strawberry pathogens, as they can infect all parts of the plant and cause severe damage or death (Rasiukevičiūtė et al. 2018).

Therefore, the objective of this study was to identify the compounds present in *M. rotundifolia* and to evaluate their effectiveness against *B. cinerea*. Similar studies have been conducted previously; however, our research aims to provide knowledge about the mechanism of action of essential oil constituents. This is important because the composition of essential oils varies depending on factors such as the geographic origin of the plant, climate conditions, developmental stage, and harvest season. In the future, these findings could contribute to the development of biological treatments against phytopathogens that affect crops, as well as to establishing a method that facilitates food preservation without any negative repercussions on stored food.

MATERIAL AND METHODS

Plant material. The aerial parts of *Mentha rotundifolia* were harvested in March 2023 from the region of Taza, 30 km northeast of Jijel on the Mediterranean coast (36°47'34''N 5°39'53''E). The collected samples were air-dried in shadow at room temperature, 20–25 °C, for two weeks and then were stored in glass boxes for further use. Taxonomic verification was conducted according to the flora of North Africa at Tela Botanica: *Mentha* × *rotundifolia* (Tela Botanica 2025)

Extraction of essential oils. The hydrodistillation apparatus type combined with Clevenger system is recognised by the European Pharmacopoeia (2008). It has the advantage that the water in which the plant materials are immersed can act as a barrier, protecting the oils from overheating and preventing the deleterious effect of the temperature on the aromatic properties of the essential oils (Ferrentino et al. 2020). The principle of the process, according to the previous author, consists of immersing the plant material in a water bath and heating it until it reaches the boiling point. When heated, plant materials release their essential oils, which are then carried through the condensation tube with water vapour. The cooling effect of cold water allows the aromatic water (hydrolat) to separate from the oils by density difference. Despite the fact that the apparatus allows for the recycling of the distillate's aqueous phase in the boiler. A sample of 100 g was placed in a 2 L round-bottom flask with 1 000 mL of distilled water, subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus (1928) to produce essential oil *M. rotundifolia*, and the essential oil was preserved in an amber flask at low temperature (4 °C) until further analysis.

Yield calculation. The essential oil yield is defined as the ratio of the essential oils obtained to the dry mass of the plant material to be treated (Kaid Slimane 2004). The following formula calculates the return as a percentage:

$$R = P_2/P_1 \times 100$$

where: R – yield of essential oils (%); P_2 – mass of essential oils (g); P_1 – mass of sample (g).

GC–MS analysis. Gas chromatography/mass spectrometry (GC/MS) analysis was performed on the isolated volatile compounds using a Shimadzu GC/MSQP2010, quadripole El 70ev type (Shimadzu, Japan). The oven temperature was set to 55 °C for 4 min, and then increased to 120 °C

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at a rate of 3 °C per minute for 5 min, and then increased to 240 °C at a rate of 5 °C per minute for 5.33 min. The injection port temperature was 250 °C (split ratio: 20.0), and the carrier gas was He with a flow rate of 1.0 mL/min. The sample (1 µL) was injected in the split mode. The following were the mass spectrometer conditions: the ion source temperature was 200 °C, and electron ionisation mass spectra were collected over the mass range (40–350 m/z). The compounds were identified by comparison of their mass spectra with those of NIST02 library data of the GC/MS system, by comparing retention times and mass spectral data with standard compounds injected under the same chromatographic conditions, as well as their retention index relative to a standard mixture of n-alkanes (Hendel et al. 2019).

Pathogen agent. Fruit samples were taken from the strawberry crop variety 'Savana' in two greenhouses in the area of Tassoust-Jijel. The greenhouses used are made from plastic film and have a use period of two years. Additionally, the fertilisation system and quality of the fertiliser were the main problems we discovered, which had a negative impact on the growth of the plants' anterior. The plants are deteriorating due to the greenhouse's temperature fluctuations, which are significant (50 °C during the day and 25 °C at night). If the air is too hot and dry, insects will proliferate quickly, while in too cool and humid conditions, fungal diseases can threaten the crop. The choice of the crop was made based on its economic importance, besides its high production and consumption in the region of Jijel and the whole country. During the spring 2022–2023 production cycles, the simples have a high incidence of fungal diseases. All samples were stored in sterile paper bags, putting them in a cooler until they were transported to the laboratory.

The infected parts of fruit were transferred onto a Petri dish with potatoes dextrose agar (PDA) medium and incubated for 6 days at 25 °C. Small agar pieces (5 × 5 mm) were cut from the grown mycelium, transferred to the centre of a Petri dish containing fresh PDA. After incubation, the isolate obtained is purified by direct transplanting on the PDA medium until a pure strain is obtained. The technique involves taking a small square (mycelian disc containing spores) from the colony. After that, we transferred the discs to new Petri dishes that contained PDA culture medium (Botton et al. 1991). The macroscopic examination determines the following characteristics: growth rate, thallus texture and colour, culture reverse colour, and average changes of the isolate were daily examined. Regarding the microscopic identification, it is based essentially on the morphological study of mycelium (absence or presence of partitions, colour of mycelial filaments, etc.) and spores (shape, colour, size, texture of walls, etc.). However, the identification was achieved by placing a drop of lactophenol on a clean slide with the aid of a mounting needle, where a small portion of the colony from the representative fungi cultures was removed and placed in a drop of lactophenol. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The identification of the fungi at a genus level was carried out by comparing the morphological characteristics of the culture (texture and measurements of shape and size of anamorphic structures, as well as conidia, conidiophore and sclerotia, etc.), which were compared using taxonomic identification keys by Barnett and Hunter (1998); Davet and Rouxel (1997). For both short-term and future inoculations, the colonies are stored in sterile distilled water at pH 6.5 and 4 °C to ensure their viability (McGinnis et al. 1974).

Essential oils concentrations and controls. After preliminary tests, in addition to pure essential oils, three concentrations of essential oils were used and prepared by diluting each time in the solvent of ethanol 95%, the successive volumes of 10, 30 and 60 mL of essential oil getting respectively the following concentrations of pure essential oils (EOs) 100%, 10%, 30% and 60%. One negative witness was prepared, constituted of ethanol (Antonov et al. (1997).

Biological activity tests. The antifungal properties of essential oils were evaluated, which included their volatile effects on mycelium growth and their inhibitory effect on *B. cinerea* using the method of Tatsadjieu et al. (2009).

Determination of mycelial growth. *B. cinerea* was performed by the agar medium assay. PDA medium with different concentrations of essential oils (10%, 30%, 60%, and 100%) was prepared. Then, (1 mL) of each concentration was transferred to each Petri dish. About 16 mL of the medium was poured into Petri dishes. Then inoculated at the centre with a mycelial disc (6 mm in diameter); Three replicates were conserved for each

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treatment. Additionally, controls (without essential oils) were inoculated following the same procedure. Petri dishes were incubated at 25 °C for 6 days. The mean radial mycelial growth was determined by measuring the diameter of the colony in two directions at right angles. Then, the negative control consists of PDA mixed with sterile distilled water, while the positive control consists only of the culture medium.

Evaluation of the mycelial growth inhibition rate. The effect of essential oils vapours against the tested strains (*B. cinerea*) was also estimated using the volatile activity technique as described by Neri et al. (2006) with slight modifications. We used this method to exploit the properties of the volatile phase of the essential oils. This method consists of transplanting two disks (the mycelial disc of *B. cinerea* and the sterile filter paper disc, imbibed with different concentrations of essential oils of *M. rotundifolia*; each disc has a diameter of 6 mm) in two separate boxes. *B. cinerea* was introduced into the centre of a Petri dish containing the PDA medium. Sterile filter paper imbibed was introduced into another Petri dish (in the centre) contains PDA medium; an assembly is made by superposing the two boxes. The paper disc imbibed at the bottom and the mycelial disk at the top. The junction between the two boxes is ensured by layers of parafilm to avoid any loss of volatile. The conditions of culture are identical to those of the confrontation by direct contact on culture medium, which the direct confrontation "Fungal strain/Essential oils" is a technique that consists of diffusing EOs in the Petri dish containing agar medium and then inoculating it with the tested fungal isolate. The positive witness is formed by superimposing two boxes, the top one containing a pellet of the isolate tested, while the bottom contains only PDA medium. However, the negative control (ethanol) was carried out by using the same manipulation, but the sterile Watman paper disc was soaked only with ethanol (1 mL) and incubated at 25 °C for 6 days. Three repetitions were performed for each concentration and each control. Mycelium growth diameters were noted daily, and data were expressed as percentage inhibition of the radial mycelial growth (Thompson 1989; Abdolahi et al. 2010). Antifungal activity was measured in terms of percent mycelial growth inhibition (MGI %) calculated by the following formula:

$$MGI\ (\%) = ((d_c - d_t)/d_c) \times 100$$

where: d_c, d_t – mycelial growth diameter in control and treatment Petri plates, respectively.

To observe the direct impact of *M. rotundifolia* essential oils on mycelial growth and spores proliferation of the strawberry pathogen *B. cinerea*, macroscopical and microscopical observations are conducted every 24 h using the binocular magnifier and a light microscope.

Data analysis. The statistical processing of the test results of MGI was carried out using XLSTAT (version 5.03, 2014), which was used to plot the histograms and curves. As a statistical treatment, an analysis of variance (ANOVA) was used, followed by a Tukey multiple comparison test. The differences between treatments were considered significant if the P-value < 0.05.

RESULTS AND DISCUSSION

Yield in essential oil and chemical composition of *Mentha rotundifolia*. The essential oils obtained from *M. rotundifolia's* extraction by hydrodistillation yielded 1.37%. The essential oils obtained were liquid, the colour was light, clear yellow, and the odour was fresh, characteristic and persistent. *M. rotundifolia* harvested in our region (Jijel) is higher than Tunisia's 1.26% (Riahi et al. 2013) and Morocco's 1.17% (Kasrati et al. 2015), but lower than Algeria's 1.8% (Brada et al. 2007). The highest yield 4.33% was obtained from *M. rotundifolia* from Morocco (Derwich et al. 2010); however, low levels were obtained by hydrodistillation and microwave from Naciria (60 km east of Algiers) 0.22% and 0.13%, respectively) (Haddache et al. 2017). *Mentha rotundifolia* of Corsica produced very low yields 0.08–0.10% (Sutour et al. 2008). The low value obtained can be attributed to several intrinsic (growth stages and plant material age) and extrinsic (plant as the period and middle of harvest, cultural practices, and all soil and climate conditions, drying and extraction methods) parameters (Benayad 2008). Furthermore, abiotic or physicochemical characteristics have an impact on essential oils yield, such as humidity, temperature, and sunshine time.

Regarding the analysis of *M. rotundifolia* EOs, sixty-seven compounds were identified and the main constituents were pulegone 39.21%, ß-cubebene 11.72%, caryophyllene 9.18%, dlimonene 4.7%,

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Table 1. The main chemical composition of *Mentha rotundifolia* essential oils (EOs) (analysed June 27, 2023)

No.	Rt	Area	Identified compounds	%	Base m/z
1	4.952	10 624.717	1R-α-pinene	1.48	104.05
2	6.657	15 122.041	myrtenol	2.1	93.10
3	6.798	7 653.332	β-phellendrene	1.06	93.05
4	7.572	15 863.440	myrcene	2.21	93.10
5	8.832	33 781.311	D-limonene	4.7	68.05
6	9.620	17 167.452	β-ocimene	2.39	93.05
7	10.244	8 960.928	α-terpinene	1.25	93.05
8	14.655	15 067.260	octen-1-ol, acetate	2.1	43.00
9	26.354	66 019.651	caryophyllene	9.18	93.05
10	28.196	8 722.949	α-caryophyllene	1.21	93.05
11	28.421	14 575.543	β-famesene	2.03	69.05
12	29.678	281 924.721	pulegone	39.21	67.05
13	30.048	84 248.841	β-cubebene	11.72	161.10
14	31.261	8 455.361	zingergone	1.18	79.05
15	30.896	6 831.011	α-pinene	0.95	121.10

Rt – retention time

ß-ocimene 2.39%, myrcene 2.21%, myrtenol 2.1%, ß-farnesene 2.03%. Additionally, there are other compounds in small amounts, especially: p-menth-1-en-8-ol (o p-menthol) 0.21%, 3-decyne 0.30%, etc. (Table 1) (Figure 1). The essential oils of *M. rotundifolia* leaves and stems were GC/MS analysed. The major component was identified as pulegone 39.21%, a cyclic monoterpene, including other important compounds. The yield of essential oils can vary from one plant family to another, from one species to another, and even between plants of the same species. Furthermore, this difference in EO content may be related to several factors, such as the geographic area of collection, climate, development stage, and harvest season.

According to our study, *M. rotundifolia* is rich in pulegone, which is a major component of essential oils and is considered a key factor in its antifungal properties. This study is seen as the initial one to be done on *M. rotundifolia* in the Jijel area.

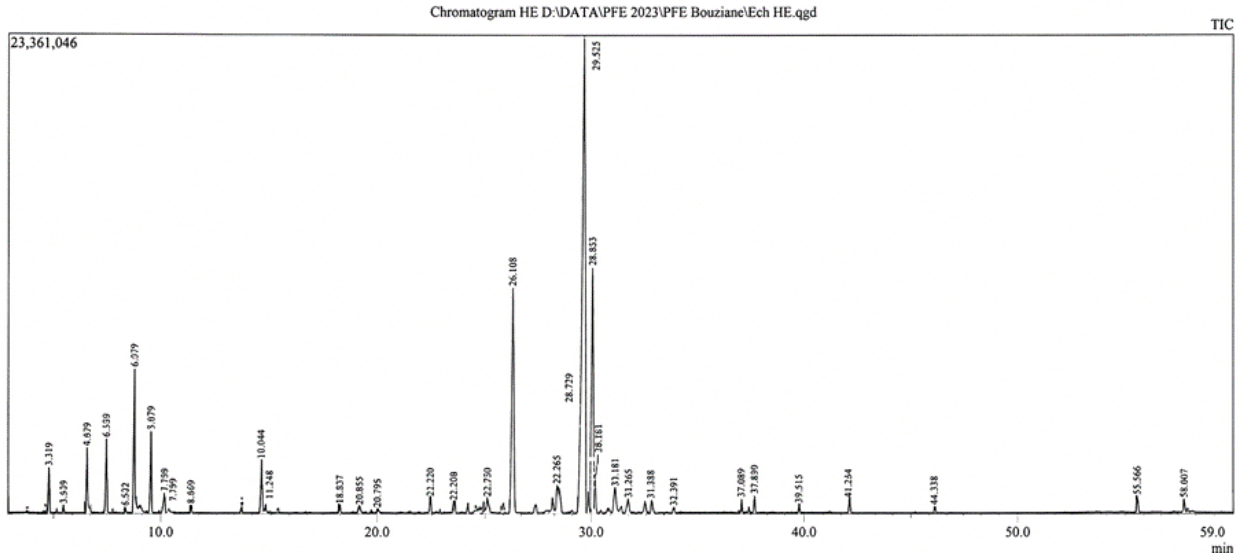

Figure 1. Chromatogram of essential oils extracted from *Mentha rotundifolia*

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The various chemotypes of *M. rotundifolia* have been clearly identified, with an oxygenated menthane derivative being the main component present in almost all chemotypes (Lawrence 2006). This is consistent with the findings of various studies on *M. rotundifolia* chemical compositions from various parts of the world containing different principal constituents, such as pulegone, menthol (Derwich et al. 2010), cis-jasmone, β-caryophyllene (Wang et al. 2013), cis-piperitone oxide (Brada et al. 2007), trans-piperitone epoxide (Brahmi et al. 2016). These results confirm previous research, which has found different chemotypes of *M. rotundifolia* growing in different parts of the world. The observed differences in yield and composition for the investigated EOs are most likely due to abiotic factors such as the climate specified in the regions of provenance of the samples, geographical factors such as altitude, and the nature of the soil. According to Riahi et al. (2013), genetic factors should not be ruled out when explaining EO chemo-variation. Furthermore, chemical differences in the oil composition of plant species were reported in relation to harvesting, season, and extraction and analysis conditions.

Description of identified fungi. On PDA medium, the isolates' colonies were warty, fluffy, and appressed, white, dirty white, greyish white to light grey or dark grey. While the reverse was hyaline to greyish. However, microscopic examination revealed that the mycelium was branched, septate, and hyaline to brown in colour. It can produce conidiophore tufts. The conidiophores were mostly straight, septate, monopodial, and branched towards the apex. Conidia were solitary, hyaline or pale brown, but in mass. The conidia observed in the microscopic fields were ellipsoidal and globose, smooth, often with a slightly protuberant hilum, and unicellular. The results obtained are similar to those obtained by Ma et al. (2018), who used different media to study the growth of *B. cinerea*. The results showed that the observation of the morphology of *B. cinerea* under an optical microscope found that the grey mould mycelium was dark grey, straight and thick, gathered into a mass or cluster and had a few branches. The mycelium had a diaphragm approximately 20 to 25 μm in length. Conidia were colourless, fell off from the conidiophores after the conidia matured, and were oval or round in shape (Figure 2).

Biological activity

The effect of *M. rotundifolia* essential oils on the filamentous growth of *B. cinerea*. After the incubation period, statistical analyses revealed that the average growth of strains of *B. cinerea* induced by *M. rotundifolia* essential oils varied significantly according to the essential oil's concentration (100%, 60%, 30% and 10%). In addition, our findings indicate a gradual decrease in the diameters of the colonies. Also, screening of antifungal activity by essential oils revealed the efficiency of the concentrations of (100% and 60%) against *B. cinerea* with an average of 25.90, 20.58 mm compared to both positive and negative controls (Table 2) (Figure 3). According to the study

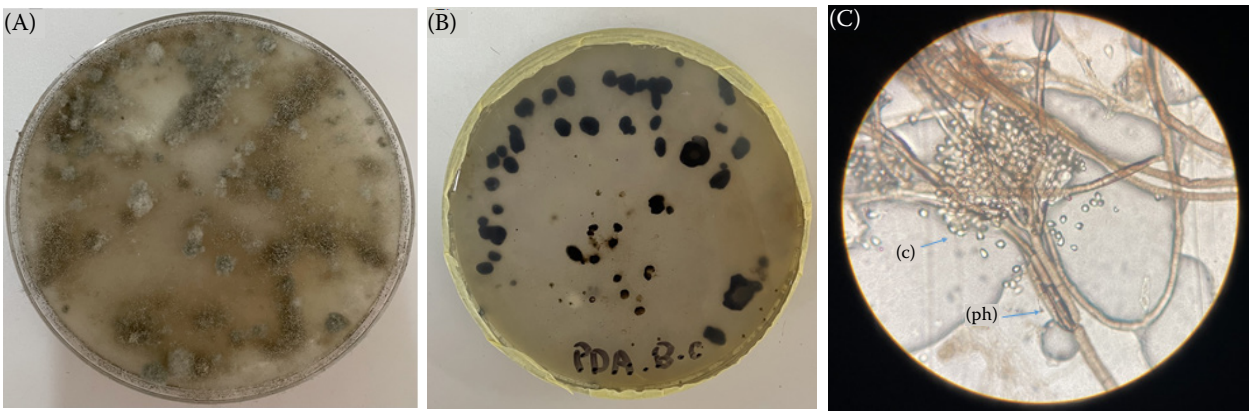

Figure 2. Morphological characteristics of *Botrytis cinerea*: (A) macroscopic aspect of the colony on potatoes dextrose agar (PDA) medium after 6 days of incubation at 25 °C; (B) sclerotia morphology on PDA medium; (C) microscopic structure ph – conidiophore; c – conidia morphology

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Table 2. The effect of different concentrations of *Mentha rotundifolia* essential oils (EOs) on the growth of *Botrytis cinerea* after 6 days from April 1 to April 6, 2023

Concentration	Average estimated	Standard error	Inferior borne (5%)	Superior borne (5%)	Homogenous groups
Positive C	56.292	3.384	56.079	56.505	A
Negative C	52.417	3.384	52.204	52.630	A
10% EO	44.208	3.384	43.995	44.421	A
30% EO	43.000	3.384	42.787	43.213	A
100% EO	25.909	3.535	25.687	26.132	B
60% EO	20.583	3.384	20.370	20.796	B

Different letters next to the values at each concentration represent a significant difference according to Tukey's HSD test ($P \leq 0.05$)

of Vilela et al. (2009), which showed that the main compounds inhibit mycelial growth of *B. cinerea*. In addition, Djekoun et al. (2024) demonstrated that the inhibition of filament growth in the strains tested could be explained by an impediment to the germination of conidia by volatile compounds in the oil. El Ajjouri (2013) states that the chemical composition of essential oils must be taken into account in its entirety. Therefore, the synergistic effect between different constituents, including minority constituents, should not be overlooked. The antifungal action of essential oils is due to an increase in the permeability of the plasma membrane, followed by a rupture of the plasma membrane resulting in a leakage of cytoplasmic content and the death of yeast (Mann et al. 2000). Also, the inhibitory effects of six monoterpenes on mycelial growth of eight phytopathogenic fungal species. All monoterpenes tested caused inhibition of mycelial growth. However, the antifungal effect depended on the specific compound and the fungal species tested were assessed by Marei and Abdelgalei (2018). Furthermore, the concentrations were assigned by Tukey's honestly significant difference (HSD) test to the same group A, indicating that there is no significant difference in growth inhibition between the negative control, positive control and 10% and 30% while, the concentrations 60% and 100% were ranked into group B with significant differences with the other ones.

Percentage inhibition of *M. rotundifolia* essential oils. Regarding minimum inhibitory effect, it should be noted that the sensitivity of microorganisms to essential oils action varied greatly depending on the method of application. The concentrations of *M. rotundifolia* essential oils were tested at 10%, 30%, 60%, and 100%. Based on the statistical analyses, the growth inhibition effect of *M. rotundifolia* essential oils on *B. cinerea* strains is concentration-dependent. Higher

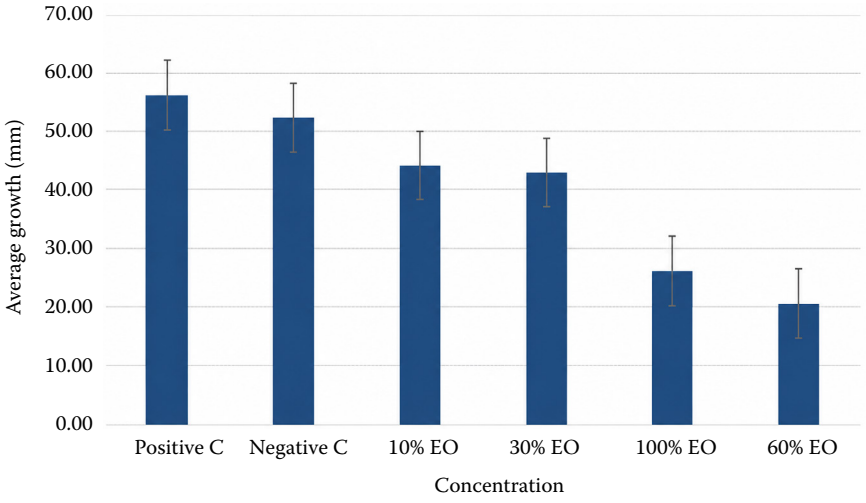

Figure 3. Average growth of *Botrytis cinerea* strains treated with *Mentha rotundifolia* essential oils (EOs), in function to various concentrations and various periods

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Table 3. The growth inhibition effect of *Mentha rotundifolia* essential oils (EOs) against *Botrytis cinerea* strains after 6 days from April 10 to April 15, 2023

Concentration	Average estimated	Standard error	Inferior borne (5%)	Superior borne (5%)	Homogenous groups
60% EO	60.839	3.291	60.632	61.046	C
Negative C	52.417	3.291	52.209	52.624	A
100% EO	48.079	3.291	47.872	48.286	C
30% EO	15.566	3.291	15.359	15.773	B
10% EO	14.455	3.291	14.248	14.662	B

Different letters next to the values at each concentration represent a significant difference according to Tukey's HSD test ($P \leq 0.05$)

concentrations (60% and 100%) inhibited growth more than lower concentrations (10% and 30%). The concentrations of 60% and 100% were highly significant against *B. cinerea* strains ($F = 17.516$, $P < 0.0001$) with inhibition percentages of 60.83% and 48.07%; the inhibition percentage of 60% concentration is higher than the one of 100%, referring to the injection of only 0.25 mL of EOs (for 100% concentration). Indeed, *M. rotundifolia* essential oils were fungitoxic by contact, in contrast to vapours, which were fungistatic by fumigation (Aouadi et al. 2021). The differences in the polarities and volatilities of the individual essential oil components, according to Cox et al. (2001), can explain the variability in essential oil efficacy related to the mode of application (contact or fumigation). Hydrophilic polar constituents mix and diffuse easily in aqueous media, resulting in greater effects in the direct contact method. These findings rank both concentrations in the same group C according to Tukey's HSD test, which allowed for the identification of homogeneous groups, indicating significant differences between the negative control and the various essential oils concentrations, while non-significant differences between the two previous concentrations. Furthermore, the other tested concentrations (10% and 30%) according to Tukey's HSD test, were ranked into group B with no significant differences between them but a highly significant difference compared to the negative control, with inhibition percentages of 14.45% and 15.56% (Table 3) (Figure 4 and 5). The hydrophobic nature of the hydrocarbon constituents of EOs allows them to be inserted into the lipid layers of the microbial cell membrane and mitochondria, disrupting structures and making them more permeable. As a result, leakage of ions and other cell components can occur (Sikkema et al. 1994; Montenegro et al. 2020; Carson et al. 2002).

The importance of essential oils of *Mentha* against phytopathogenic agents has been studied by numerous researchers, who pointed out that the

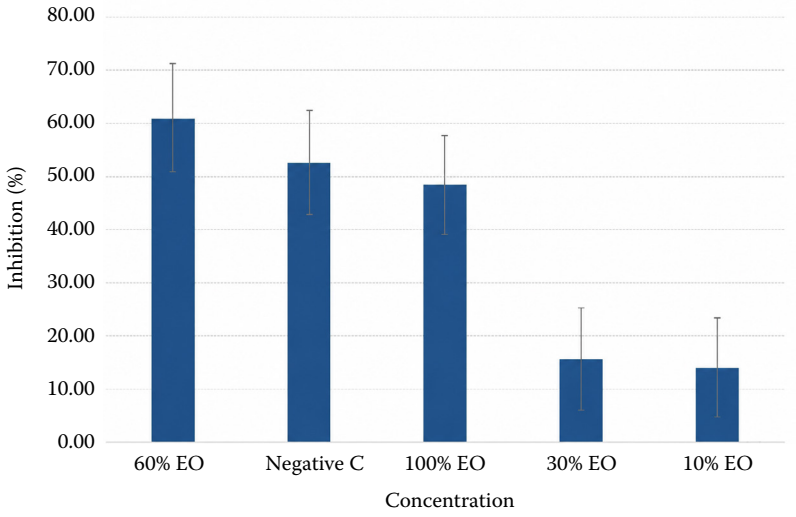

Figure 4. Inhibition percentage induced by various concentrations of *Mentha rotundifolia* essential oils (EOs) on the growth of *Botrytis cinerea*

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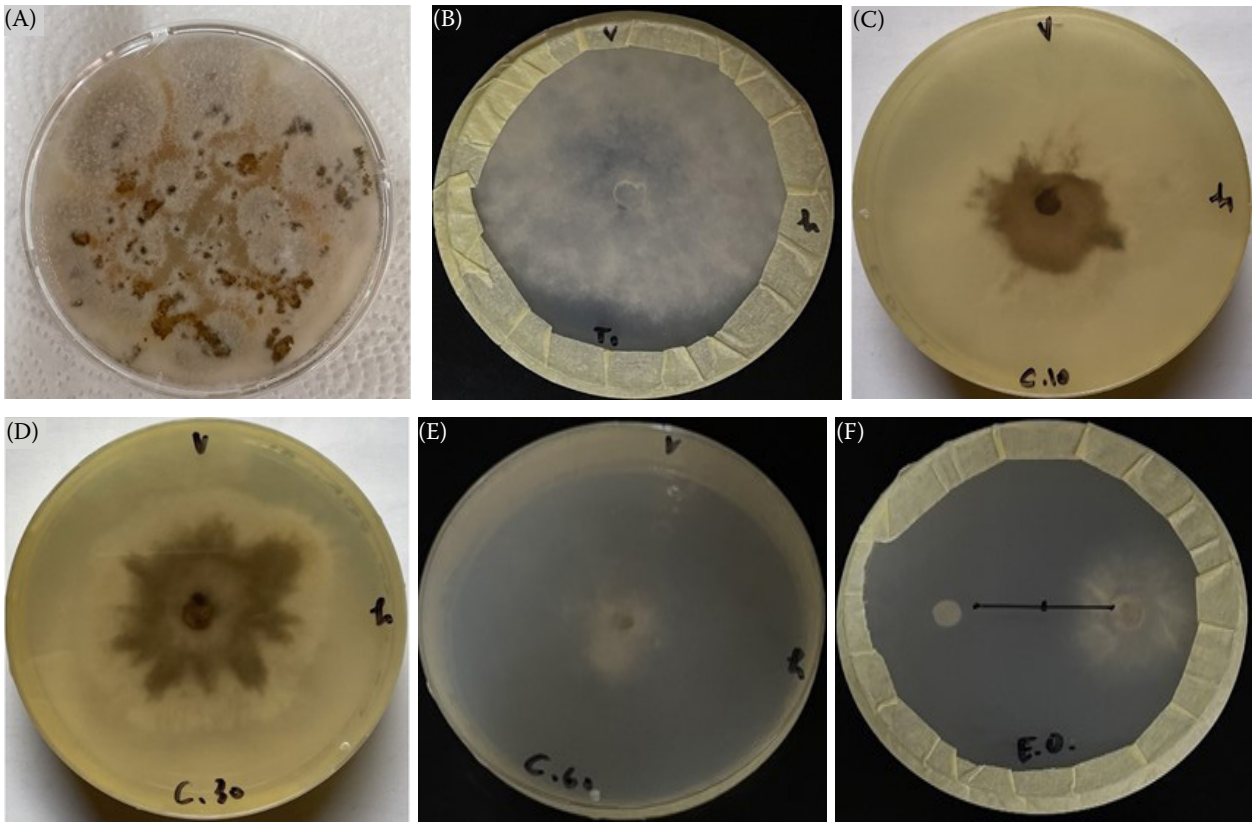

Figure 5. Inhibition effect of different application concentrations of *Mentha rotundifolia* essential oils (EOs) on the mycelial growth of *Botrytis cinerea* after 6 days of incubation: (A) positive control, (B) negative control, (C) concentration 10%, (D) concentration 30%, (E) concentration 60%, (F) concentration 100%, respectively

effect of EOs vapours could be associated with the formation of the fruit surface coating that modifies gas permeation and decreases respiration rate and water loss (Santoro et al. 2018). Similar findings were reported by Badawy et al. (2016), who observed that the use of EOs containing thymol

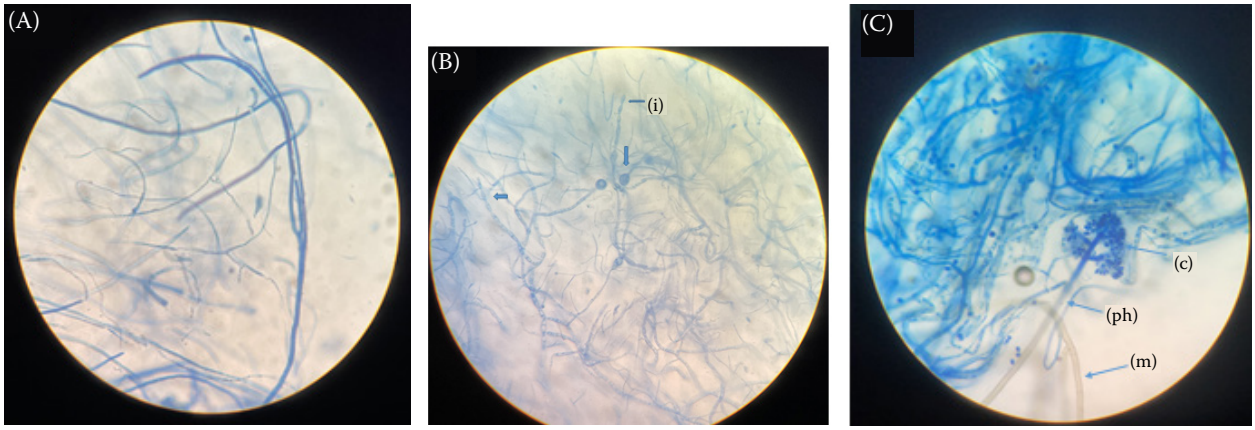

Figure 6. The appearance of morphological modification in the structure of *Botrytis cinerea*: (A, B) mycelium became siphoned, and membrane deformation occurred, (C) untreated positive control

i – macrovacuolization of mycelia after treatment with essential oils (EOs) concentrations of *Mentha rotundifolia*; c – conidia, ph – conidiophore; m – septate mycelium

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0.02% or geraniol 0.04% increased catalase (CAT) activity and reduced polyphenol oxidase (PPO) activity and peroxidase, thus promoting prolongation of the shelf-life and preserving the quality of strawberries during storage.

The microscopic observations revealed that the effect of *M. rotundifolia* essential oils caused a mycelial growth inhibition of the pathogen of strawberry, *B. cinerea*. In addition, the essential oils concentrations completely suppressed the germination of spores of *B. cinerea*, which were found to be involved in the degradation of walls and membranes of plant pathogen cells. Furthermore, the mycelium appears emptied and siphoned, and we noted the presence of macro vacuoles; Similarly, the results obtained by Aouadi et al. (2022) affirm that the treated hyphae appeared thinner and were characterised by an irregular surface, the absence of septation and the presence of macro vacuolisation (Figure 6). Several studies have highlighted the inhibitory action of essential oils on the germination of fungal spores (Vitoratos et al. 2013; Farzaneh et al. 2015), whereas the mechanism of action remains ambiguous and misunderstood. Nonetheless, Carson et al. (2002) and Pei et al. (2020) affirm that their activity flows from their ability to disrupt the structure of fungi cell membranes, which explains the results obtained about the antifungal activity of *M. rotundifolia* against *B. cinerea* that can probably be attributed to the majority of oil compounds.

CONCLUSION

According to our study, *M. rotundifolia* is rich in pulegone with a value of 39.21%. Pulegone, a major component of essential oils, is considered a key factor in its antifungal properties. This study is seen as the initial one to be done on *M. rotundifolia* in the Jijel area. Furthermore, the bioassay results show that the essential oils of *M. rotundifolia*, which are rich in bioactive molecules, are extremely effective against the phytopathogenic fungus. Indeed, it completely inhibited the mycelial growth of the tested fungal strains and completely stopped spore germination by inducing big morphological changes that led to their explosion. The effect/concentration response for phytopathogenic fungi was significant. As a result, our findings support the use of *M. rotundifolia* essential oils as a biological control against plant diseases. We recommend using essential oils as a new ideal and innovative strategy with ecological properties to protect plant crops. However, their practical application as safe alternatives to the synthetic compounds necessitates further research to develop formulations that improve their effectiveness and stability; this is according to their application. There are multiple ways to apply *Mentha* essential oils, including direct contact with pest, ingestion, inhalation (or fumigation), or spraying. In addition, it is recommended that the various biochemical molecules identified *in vitro* be tested on various biological models with the view of using them for therapeutic and food preservation purposes. Further, we suggest that future research should focus on evaluating the effects of *M. rotundifolia's* compounds individually or combining them with other bioactive substances to achieve more effective control. It would also be important to determine the mechanisms of essential oils on reproduction, growth of pathogens, and behaviour of insects. Furthermore, the environmental influence of essential oils on non-targeted biodiversity.

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