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Evaluation of Antifungal Potential of *Mentha pulegium* Essential oil in Biological Control Against the Pathogen of Inflorescence Rot Disease of Date Palm (*Mauginiella scaettae)*

Hammia Hadjra [1,*], Bouatrous Yamina [1], Kriker Soulef [1], Ramazan Erenler[2]

[1] *Department of Nature and Life Sciences, Faculty of Exact Sciences and Nature and Life Sciences, Mohamed khider University, 07000;*

[2] *Department of Chemistry, Faculty of Art and Science, Tokat Gaziosmanpasa University, Turkey.*

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Abstract

In desert environments, the date palm is a vital component of the oasis ecosystem. Despite its importance to the local community and the national economy, date palm agriculture in Algeria faces a number of challenges. One of the biggest obstacles is infectious diseases, such as the fungus *Mauginiella scaettae*'s date palm inflorescence rot. While fungicides play an important role in controlling this disease, their use can have negative consequences for human health and the environment due to the presence of residues. In the context of this growth, there is an increasing interest in moving from pesticides to ecologically benign disease management methods. Thus, it is necessary to study innovative methods for the effective and safe control of inflorescence rot. Due to their antifungal qualities, it appears that using the essences of some aromatic and medicinal herbs is one of the most efficient methods for preventing fungal illnesses. In this regard, the latest research evaluates the efficacy of an alternative technique to synthetic fungicides, specifically the examination of *Mentha pulegium* essential oils' antifungal activity "*in vitro"*. Therefore, the purpose of this research consisted of the identification of chemical components involved in *Mentha pulegium* as well as investigation of its possible antifungal activity against *Mauginiella scaettae*. *In vitro* testing was done to determine the antifungal effects of volatile oil at selected doses (0.25, 0.5, and 1) µl/ml against *Mauginiella scaettae*. The obtained data show a yield of 0.99 ± 0.041% for the essential oil. Seventeen components were identified in the essential oil studied. Isomenthone (19.31%), Thymol (11.39%), Piperenone (5.37%), Piperitone (3.04%), and p-Menthan-3-one (4.76%) are the principal constituents detected. The results demonstrated a considerable influence on mycelial growth, with inhibition rates ranging from 92.90 ± 12.28% to 100%. In addition, it was discovered that the essential oil concentration of 0.5µl/ml totally eliminated pathogen growth. This concentration was also the MIC (minimal inhibitory concentration) of *Mauginiella scaettae*. Our investigation revealed that *Mentha pulegium* essential oil possesses antifungal efficacy against the pathogen examined. It can, therefore, be utilized as an alternate antifungal agent.

Keywords: Antifungal activity; GC-MS analysis, Inhibition, *Mauginiella scaettae*; *Mentha pulegium*.

1. Introduction

In the Algerian Sahara region, the production of palms is considered one of the essential agro-activities of the country, date palms being the key plant in the socio-economic context of the region (Benziouche and Cheriet, 2012). Algeria's agriculture is based on the date palm sector, which constitutes the country's second-largest export after hydrocarbons and the primary means of livelihood for those who live in desert oasis regions (Abdelmalek, 2023). Algeria is distinguished by several date varieties, which are characterized by their nutritional value and high yield; their production in 2019 was estimated at 1.4 million tons, and among the most important of these varieties, we cite the Deglat Nour, which accounts for 49% of total date production and is regarded as the most demanded variety locally and internationally (AlFaris *et al*., 2022).

Yet the tree is plagued with numerous pathogens and parasites. One of the most serious fungal diseases is the inflorescence rot (Khamedj), which can infect a palm's male or female in regions with favorable climatic circumstances, such as excessive humidity. Khamedj disease is an important factor limiting productivity. Disease losses are around 2-15%, but can exceed 50-80% in severe epidemics. (Alvanipour *et al.*, 2020; Bouhlali *et al*., 2021). Significant yield losses attributable to this disease contribute to its economic significance and might even result in genetic degradation (Bounaga and Djerbi, 1990; Dakhia *et al*., 2013). Inflorescence rot (Khamedj) has been documented in North African date-growing regions (Egypt, Sudan, Libya, Tunisia, Algeria, and Morocco), and its proliferation has been detected not only in North Africa but also in other regions where palms are cultivated, such as Iraq, Palestine, Mauritania, Saudi Arabia, and Italy (Chabrolin, 1928; Hussain, 1958; Michael and Sabet, 1970; Munier, 1955; Al- Ani *et al.*, 1971;). In contrast, Alvanipour *et al.* (2020) demonstrate

* Corresponding author. e-mail: hadjra.hammia@univ-biskra.dz.

that this malady is among the most major date palm diseases in the Iranian province of Khuzestan, where a substantial number of palm trees are infected. In fact, it was caused by the pathogen *Mauginiella scaettae*, which Cavara discovered in Libya in 1925 for the first time (Hussain, 1958; Al-Ani *et al*., 1971; Abdullah *et al*., 2005). Several control measures have been established to combat this disease, including the elimination of affected inflorescence portions and their quick burning after harvest, as well as the use of several fungicides on palms (Carpenter and Elmer, 1978). Due to toxicological and ecotoxicological hazards, some pesticides might also have negative effects affecting people as well as the natural world. Considering all these negative effects, it is necessary to investigate alternatives to chemical control. In fact, a great deal of research has been carried out to create novel alternatives for controlling pathogen-caused diseases (Youssef *et al*., 2012).

Due to their antibacterial activity, which is reliant on their chemical composition, especially the type of their primary volatile components, the natural product of plant extracts, like as essential oils, have been of great importance as biocontrol agents (Caillet and Lacroix, 2007; Bouhlali *et al*., 2021). This research has focused on the utilisation of a wide variety of antifungal agents, including natural compounds. Owing to its antifungal qualities, the use of plant essences derived from particular therapeutic and aromatic herbs looks to be one of the highly efficient methods for combating this condition. On the basis of microbiological and antifungal properties, it was determined that *Mentha pulegium* essential oils contain diverse naturally occurring compounds with biological activity (Belghazi *et al*., 2002). It is widely dispersed around the world, particularly in Europe, the Middle East, Asia and North Africa. Historically, it was utilized for its antiseptic, anticholera, antitubercular, and anti-inflammatory properties. In addition, the essential oil has antifungal, insecticide, antiparasitic, antispasmodic, and antioxidant properties (Teixeira *et al*., 2012; Marzouk *et al*., 2008). Yet, the essential oil's antifungal effectiveness against the fungus responsible for date palm inflorescence rot has never been investigated. Therefore, identifying their chemical components as well as the study of the antifungal properties of *Mentha pulegium* essential oil towards *Mauginiella scaettae* were the aims of our current research, with the objective of discovering active natural compounds for a potential biological control of this disease.

2. Materials and methods

2.1. Plant material

Mentha pulegium herb has been gathered in March 2019 from the Besbes region, South East Algeria (34°09′00″N and 4°59′27″ E). This one was identified at the University of Biskra's Centre for Scientific and Technical Research on Arid Regions (CRSTRA). The *Mentha pulegium* species' aerial parts (stems and leaves) were air-dried and preserved until extraction in sterile paper sacks.

2.1.1. Extraction of Menthe pulegium essential oil

Hydrodistillation was used to extract the *Mentha pulegium* oil using the Clevenger apparatus. A Pyrex glass bottle containing 100 g of the dry plant material was used to extract the oil, for three hours with 1000 ml of distilled water. Once obtained, the gathered oil was kept out of the light at a temperature of 4 °C (Ismaily *et al*., 2014).

2.1.2. Determination of the Mentha pulegium essential oil yield

Afnor standard (1986) was used to define the essential oil yield. It really is estimated using the following formula in percentage form (Afnor, 1986).

$Yeo = Weo/W_{dh}.100$
Yeo (%): essential oil yield
Weo: weight of the essential oil obtained (g)
W_{dh}: weight of powdered plant material (g)

2.1.3. Gas chromatography- mass analysis

Mass Spectrometer with an ISQ single Quadrupole attached to Trace 1310 Gas Chromatograph was utilised in the analysis (Thermo Fisher Scientific, Austin, Texas). The initial temperature for the treatment was 60 °C for 6 minutes, followed by a ramp to 230 °C at a rate of 2 °C/min, and then 30 minutes at 230 °C. Both the detector and ion source temperatures were 250 °C. We filtered the sample through a disposable syringe filter (0.22 µm). 1µl was injected into the split-less model. The analyzed sample was separated using Thermo TG-WAXMS GC column (60 m x 0.25 mm ID x 0.25 µm) with 1.2 ml/min of Helium being used as the carrier gas. The range of the mass spectral scan has been adjusted between 55 and 550 (amu). Peaks were identified using NIST Demo components Wiley7, Wiley 9, redlip, mainlip, and WinRI, as well as Wiley7, Wiley 9, redlip, and mainlip (Adams, 2001).

2.2. Collection and purification of pathogens

The 2 cm long fragments of the contaminated inflorescence have been sterilized for 3 minutes using 2% sodium hypochlorite, followed by 3 rinses in sterile distilled water for 3 minutes, then dried on autoclaved filter paper. The samples were subsequently dried on sterile pads then transported in sterilized humid filter paper placed in Petri plates, three pieces in each dish. They would be incubated for seven days under dark conditions at 25±2°C. When the different colonies were clearly distinct, they were re-inoculated repeatedly into novel Petri plates that contained medium PDA to isolate pure colonies. Following obtaining pure strains, macro and microscopic examinations have been carried out to identify them (Abdullah *et al*., 2005; Rattan and Al-Dboon, 1980).

2.3. Evaluation of antifungal activity of essential oil

The activity of the essential oils was determined using the directed contact technique by diluting the essential oil with Tween 20 (0.1%) (v/v) followed by adding it to 20 ml PDA. Three concentrations were obtained: 0.25, 0.5, and 1 µl/ml PDA. 20 ml of PDA medium was placed in each Petri plate, and after that 1 ml of each concentration has been added, homogenisation of the culture medium was carried out by mixing for 5 minutes. Once the medium had solidified, a mycelial disc with a diameter of 0.5 cm was cut from the edge of a 7-day-old culture and placed in the center of each PDA plate. The plates were then incubated in the dark at 25 ± 2°C for 7 days. The negative control consisted of 20 ml (PDA + Tween 20) but no essential oil. Triplicates were utilised for both the examined and control

oils (Remmal *et al*., 1993). The mean of two diameters perpendicular to each other across the middle of the box was used to calculate the daily radial spread of cultured explant. The following formulas were used to determine the inhibitory potential (Leroux and Credet, 1978) and mycelial growth rate (Howell, 2003) for each concentration.

- Mycelial growth inhibition rate (MGI)

MGI = [(C - c) / C] x 100
MGI (%): Mycelial growth inhibition rate;
C: the control colony's diameter in (cm);
c: the experimental colony's size in (cm).

- Rate of mycelial growth

To measure colony growth, the following formula was applied:

MGR = Dc-dex / 2
MGR: mycelial growth rate (cm)
Dc: is the colony's diameter (cm)
dex : the diameter of explant (cm)

2.4. Determining the minimum inhibition concentrations (MIC)

The lowest concentration of an essential oil at which no growth is evident to the naked eye is referred to as the "minimum inhibitory concentration." The minimal inhibitory concentration was calculated based on the complete absence of microbiological growth at the various essential oil doses examined (MIC) (Remmal *et al*., 1993).

2.5. The statistical study of the experimental results

The acquired data were subjected to one-way analysis of variance (ANOVA), and any significant differences between doses of essential oil tested were identified using the Newman-Keuls test. The means are also presented in the form of (mean ± SD) with $p < 0.05$ as the level of significance. This analysis was performed with the XLSTAT 2014.5.03 analysis software.

3. Results and Discussion

3.1. Essential oil Yield

Essential oil yield from hydrodistillation was 0.99 ± 0.041 %. This extracted oil had a pleasing aroma and was pale yellow in colour (mentholated).

Our results accord with those reported by Zwaving and Smith (1971), who indicated that the yield of oils from the same species in Austria is approximately 0.95 %. On the other hand, the essential oil yield exceeds those cited by Zekri *et al*. (2013) and Hmiri *et al*. (2011) who acquired yields of (5.29–6.2%) and 3.30%, respectively.

3.2. The chemical constitution of the essential oil

Using gas chromatography-mass spectrometry (GC-MS), 17 constituents were identified in the essential oil of *Mentha pulegium* extracted from the Biskra region, which comprised the entire chemical constitution. The analyzed oil had a high concentration of oxygenated monoterpenes (82.43%), including p-Menth-4(8)-en-3-one (36.01%), Isomenthone (19.31%), Thymol (11.39%), Piperitenone (5.37%), Piperitone (3.04%), and p-Menthan-3-one (4.76%). In addition, there are smaller amounts of the following: isopulegone (0.28%), neo-menthol (0.27%), phenol, 4methyl-2-(2propenyl) (0.96%), Camphor (0.43%), and carvacrol (0.61%). On the other hand, D-Limonene (1.26 %), Ç-Terpinen (4.89 %), and Cymol (5.27 %) are the most abundant hydrocarbon monoterpenes found in this oil (Table 1 and Figure 1).

Based on the investigation's results, it appears that the *Mentha pulegium* essential oil is very different from those cited in the literature and reveals a distinct chemotype due to the presence of the three compounds p-Menth-4(8)-en-3-one, Isomenthone, and Thymol in significant concentrations and the absence of pulegone. The presence of pulegone in substantial amounts in the essential oil of *Mentha pulegium* has been demonstrated by numerous authors: Boukhebti *et al.* (2011) in Setif (Algeria) (38.815%), Stoyanova *et al*. (2005) in Bulgaria (42.9-45.4%), and Mkaddem *et al.* (2007) in Tunisia (41.8%). Moreover, in the Kazeron region (Fars Province, Iran) those same oils are rich in piperitone (38%) and piperenone (33%), while pulegone levels are low (2.3%) (Mahboubi and Haghi, 2008). However, the essential oil of the identical plant, harvested in the Boulmane area (Morocco), was analysed and found to have the piperitone chemotype (35.56%) (Derwich *et al*., 2010).

Several variables can affect the chemical characteristics and yield of *Mentha pulegium* essential oil, including geographic position, period and location of drying, temperature, methodology and duration of distillation, the type of soil, environmental factors, illness brought on by external factors, post - harvest and extraction procedures, as well as vegetative plant phase (Atailia and Djahoudi, 2015; Bergheul, 2018; Daoudi *et al*., 2016).

Table 1. Composition of *Mentha pulegium*'s essential oil (%)

Components	Retention Time	(%)
dl-Limonene	15.34	1.26
Ç-Terpinen	17.93	4.89
Cymol	19.43	5.27
Ethyl amylcarbinol	26.86	1.16
Isomenthone	31.14	19.31
p-Menthan-3-one	32.80	4.76
Camphor	34.21	0.43
(+)-Menthylacetate	35.14	0.42
Isopulegone	37.77	0.28
Thymylmethylether	38.24	4.57
neo-Menthol	41.04	0.27
p-Menth-4(8)-en-3-one	42.04	36.01
Piperitone	46.69	3.04
Piperitenone	57.32	5.37
Phenol, 4methyl-2-(2propenyl)	61.18	0.96
Carvacrol	69.54	0.61
Thymol	70.28	11.39
Total		100
Monoterpene hydrocarbons		11.42
Oxygenated monoterpenes		82.43

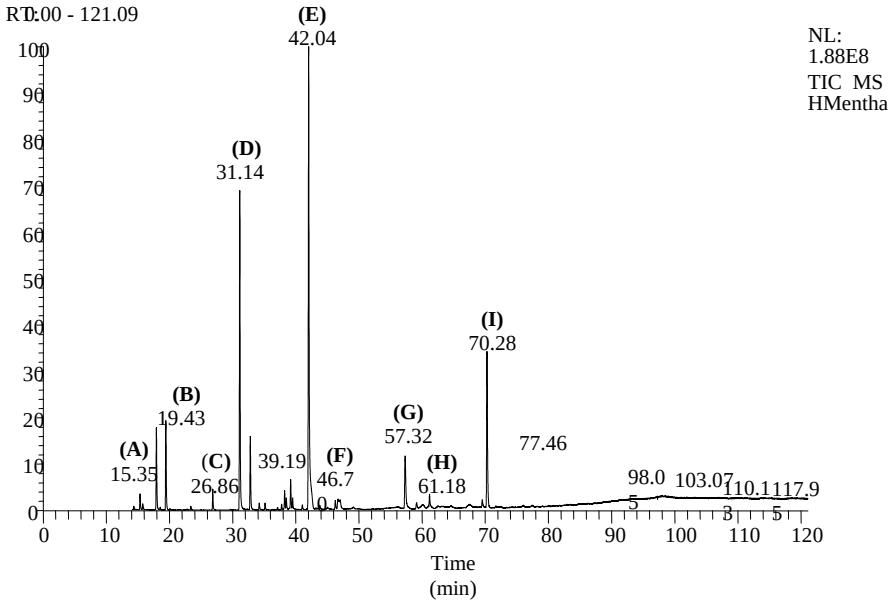


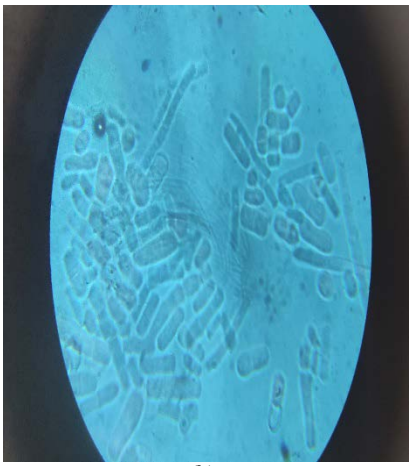
Figure1. Chromatographic profile of the essential oil *Mentha pulegium.* (A) dl-Limonene; (B) Cymol; (C) Ethyl amylcarbinol; (D) Isomenthone; (E) p-Menth-4(8)-en-3-one; (F) Piperitone; (G) Piperitenone; (H) Phenol, 4methyl-2-(2propenyl); (I) Thymol.

3.3. The essential oil's antifungal activity

3.3.1. Pathogen identification

After 7 days of growth, Mauginiella scaettae colonies on PDA were white on the front and creamy to pale brown on the back. They were 3 to 5 cm in diameter and looked powdery (Figure 2 a). According to the observed results, the mycelium consists of branched hyaline and septate hyphae measuring between 15 µm and 80 µm long and 3 to 10 µm wide. Arthroconidia are unicellular or multicellular. Mature spores are uni, bi, tri, or multicellular (Figure 2 b). Our findings validate those of Rattan and Al-Dboon (1980) and Abdullah et al. (2005) who indicated that these characteristics are typical of Mauginiella scaettae.

(a)



(b)

Figure 2. Isolation of *Mauginiella scaettae* from diseased date palm spathes. (a) The growth of the fungus on PDA. (b) The fungus' hyphae and conidia.

3.3.2. The mycelial growth inhibition rate

The three doses of oil tested (0.25, 0.5, and 1) µl/ml have a potent antifungal effect on *Mauginiella scaettae*. In fact, the tested strain is extremely sensitive to these concentrations, with the respective inhibition rates of 92.90 ± 12.28%, 100%, and 99.71 ± 0.49%.

The results of the concentration-dependent rate of inhibition of mycelial growth were then subjected to a variance analysis. According to ANOVA, there was no difference between doses, as demonstrated by p=0.43 (P> 0.05) for the non-significant difference analysis. In addition, they are placed in the same group (A) (Figure 3).

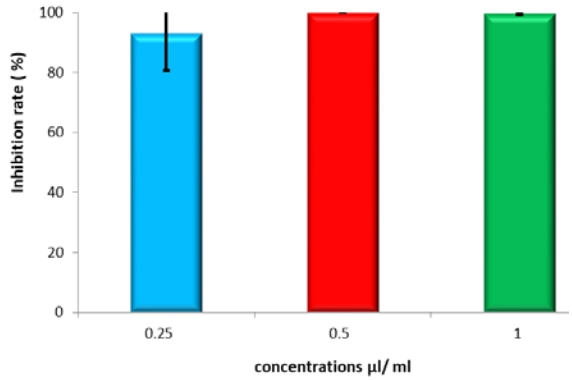


Figure 3. Growth inhibition Rate of fungi tested with *Mentha pulegium* oil

3.3.3. Growth Rate of mycelial

Regarding *Mauginiella scaettae* mycelial growth in relation to different concentrations of essential oil tested, a relatively close linear distribution was observed for three studied doses.

Complete suppression of *Mauginiella scaettae* growth was observed at concentrations of 0.5 and 1 µl/ml, to the extent that no development occurs after the application of *Mentha pulegium* oil. Furthermore, it is observed that even with the lowest concentration (0.25 µl/ml), mycelial growth progresses very slowly from the first day of the test (0.03 ± 0.06 cm) until the fifth day, when it stabilises and reaches a maximum value of (0.08 ± 0.14 cm) estimated (Figure 4).

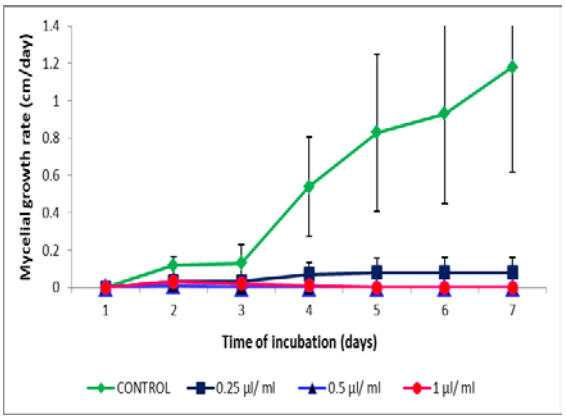


Figure 4. The variation in *Mauginiella scaettae* mycelial growth at various *Mentha pulegium* volatile oil doses.

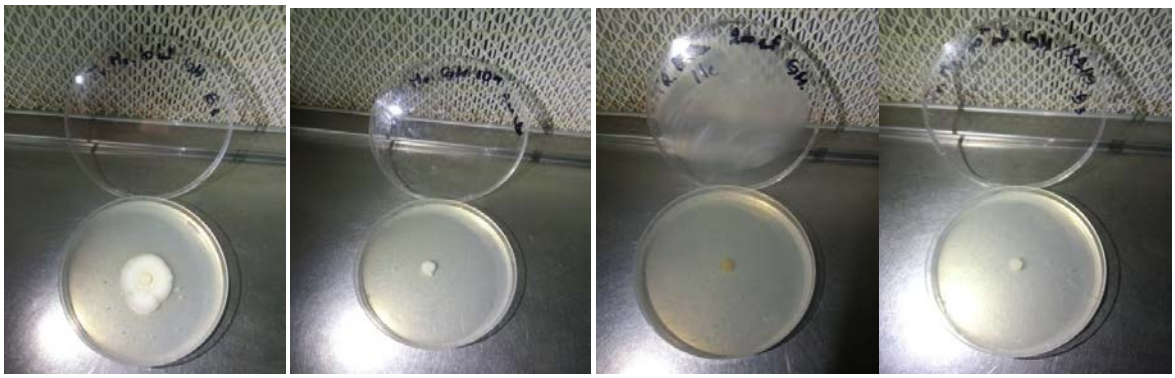
The phytopathogen *Mauginiella scaettae* has not been the subject of any published research on the effects of essential oils, so a comparison with previous research is difficult. The first mention of the fungicidal efficacy of *Mentha pulegium* essential oils is, in fact, in this study.

In light of the findings of the study, the tested essential oil examined was found to have a significant antifungal effect against the strain *Mauginiella scaettae*. In terms of the kinetics of mycelial growth, as the incubation period advanced, the growth of control was observed to accelerate. Nonetheless, an inverse relationship was reported between mycelial growth and essential oil concentration. Consequently, the mycelia diameter diminished as essential oil concentration grew. Regarding effectiveness, the *Mauginiella scaettae* strain had a greater sensitivity to essential oil concentrations than the tested species. Depending on our findings, at 0.5 µl/ ml, the *Mentha pulegium* essential oil completely inhibits the growth of the strain of fungus *Mauginiella scaettae*. These results are consistent with earlier research on the antimicrobial characteristics of the same species' volatile oil. However, Uwineza *et al.*, (2018) demonstrated a total suppression of fungal growth of *Fusarium culmorum* in 1.25 ml/l concentration. In contrast, Hmiri *et al.* (2011) found that 10 µl of *Mentha pulegium* oil completely eliminated the growth of mycelia of two fungi *Alternaria alternata* and *Penicillium expansum*. Hmiri *et al.* (2013) confirmed his findings and demonstrated that *A. alternata* was most sensitive at 156 µl/ l, whereas *P. expansum* and *B. cinerea* have been totally inhibited at 300 µl l/ l and above. Hajlaoui *et al*. (2009) mentioned the interesting antifungal activity of this oil against *Fusarium oxysporum, Trichoderma* sp., *Aspergillus flavus, Aspergillus niger, Fusarium culmorum,* and *B. Cinerea* with a volume of 10 µl. Indeed, the powerful fungicidal efficacy of *Mentha pulegium* essential oils may correlate with the high level of oxygenated monoterpene compounds and the synergistic effect with minor compounds. Several studies have shown that oxygenated monoterpenes are effective agents against microbial diseases (Lucini *et al*., 2006; Kordali *et al.*, 2003). Many studies have shown that the chemical components of *Mentha pulegium*, including phenols (carvacrol, octanol, etc.), aldehydes and ketones (camphor, etc.) alcohols (terpinen-4-ol, terpineol), are recognized to be effective antibacterial agents (Celiktas *et al.*, 2007). Moreover, even at low concentrations, the synergistic impact seen with carvacrol and thymol provides a large spectrum of antimicrobial activity (Didry *et al.*, 1993). However, Dorman and Deans (2000) assert that the observed bioactivity of essential oils is attributable not just to its major constituents but additionally to various minority elements that might collaborate synergistically or antagonistically to provide a powerful antibacterial impact. In a similar vein, a study (Cárdenas-Ortega *et al*., 2005) showed that modest amounts of piperitone completely reduced *Aspergillus flavus* strains. In fact, Lucini *et al.* (2006) demonstrated that camphor, 1,8 -cineole, linalool, and menthol slowed sclerotic development the most. Sharma and Tripathi (2006) report that the deterioration of mycelium, which appears to be devoid of cytoplasm, as well as the reduction of the rigidity and integrity of their cell walls, are brought on by the usage of essential oils. Many investigations have demonstrated that the toxicity of phenols toward molds is due to the inactivation of fungal enzymes with the SH group at their active site. Consequently, Phenolic terpenes actively bind to hydroxylamine and amine groups in the membrane

proteins of microorganisms, resulting in a disturbance of permeability and a loss of intracellular components (Farag *et al*., 1989; Celimene *et al*., 1999; Cowan, 1999; Ultee *et al*., 1999; Knowles *et al*., 2005; Lopez-Malo *et al*., 2005).

3.4. Minimum inhibition concentration (MIC)

According to observations, the fungal strain *Mauginiella scaettae* is extremely sensitive to the *Mentha pulegium* essential oils, exhibiting a 100% inhibition rate at 0.5 µl/ml concentration (Figure 5).



Control 0.25 µl/ ml 0.5 µl/ ml 1 µl/ ml

Figure 5. Photograph of *Mauginiella scaettae* mycelial growth in relation to different concentrations of *Mentha pulegium* oil during seven days of incubation

4. Conclusion

In summary, this research demonstrated the anti-fungal power of *Mentha pulegium* essential oil harvested in Besbes (Biskra - Algeria) to control the growth of *Mauginiella scaettae* strains, a pathogen responsible for date palm Khamedj disease. The findings of this research showed that the oil may serve as an important agent for controlling fungal infections. Indeed, compound analysis of *Mentha pulegium* essential oil has revealed that it contains oxygenated monoterpenes. Their effect on the fungal strain tested was very significant. The essential oil evaluated has completely limited the growth of *Mauginiella scaettae* in comparison with control. Furthermore, the results of this research have made it possible to develop a means of biological control of this major date palm pest, through the use of the essential oil of *Mentha pulegium.*

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