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Antifungal activity of essential oils of four medicinal plants on fungi isolated from potato tubers (*Solanum tuberosum*) from the El Oued region (Eastern Algerian Northern Sahara)

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Abstract

The aim of this study is to investigate the chemical composition of essential oils extracted from the aerial parts of four plants: *Mentha spicata* L, *Mentha piperita* L, *Mentha pulegium* L, and *Ocimum basilicum* L, in order to evaluate their antifungal properties against several pathogenic fungi isolated from post-harvest potato tubers. The essential oils were extracted through hydrodistillation and analyzed using gas chromatography-mass spectrometry (GC-MS). The results revealed that *M. pulegium* has the highest content of essential oils with a yield of 2.1%, followed by *M. spicata* (1.4%), *M. piperita* (0.83%), and *O. basilicum* (0.19%). *M. spicata* is composed of (-)-Carvone (41.66%) and exo-2,7,7-trimethylbicyclo[2.2.1]heptane (10.28%). (-)-Carvone is the main component of *M. piperita* (48.74%), followed by cis-dihydrocarvone (7.96%). The essential oil of *M. pulegium* is characterized by the presence of (-)-carvone as the main component with a content of 30.89%, isopulegone (23.62%). The volatile essence of *O. basilicum* contains linalool (26.16%), estragole (16.69%). The antifungal activity of the essential oils was evaluated using the direct contact method against the mycelial growth of the tested strains. The results indicated a high potential for antifungal activity against the tested strains. The essential oils of *Mentha spicata* showed a 100% antifungal effect against *Fusarium proliferatum* at a concentration of 0.90% and 0.95% for *Alternaria alternata*, *Rhizoctonia solani*, and *Wickerhamomyces anomalus*. Regarding the essential oils of *Mentha piperita*, a complete inhibition (100%) of mycelial growth was also achieved against all tested strains, at concentrations of 0.95% and 1%, and at 0.87% for *Wickerhamomyces anomalus* and 0.90% for *A. alternata*. The essential oil of *Mentha pulegium* has

Antifungal activity of essential oils of four medicinal plants on fungi isolated from potato tubers (*Solanum tuberosum*) from the El Oued region (Eastern Algerian Northern Sahara) a fungicidal inhibitory effect at a concentration of 0.97% on *Alternaria alternata* and *Fusarium proliferatum*, and at 0.95% on *Wickerhamomyces anomalus* and *Rhizoctonia solani*. Regarding the essential oils of *Ocimum basilicum*, a complete inhibition (100%) of mycelial growth of fungal strains was achieved at a concentration of 0.97% for all strains except *Wickerhamomyces anomalus*, for which the fungicidal effect was obtained at a concentration of 1%. The obtained results, pave the way for the utilization of essential oils as an alternative to chemical fungicides.

Keywords: : Potato, medicinal plants, phytopathogenic fungi, essential oils, antifungal activity.

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1. Introduction

The potato (*Solanum tuberosum* L.) is one of the most important crops worldwide and ranks fourth among all food crops in total production (FAO, 2022). However, potatoes are susceptible to certain diseases caused by various fungal pathogens, such as *Colletotrichum coccodes* (Johnson *et al.*, 2018), which causes black dot disease, and *Helminthosporium solani* Dur, which causes silver scurf (Massana-Codina *et al.*, 2021).

These pathogens result in significant post-harvest losses. To address these issues, chemicals are commonly used, but they come with limitations and numerous drawbacks, such as pollution, phytotoxicity, disruption of biological balance, and particularly the risk of developing fungicide-resistant strains (Arias-Rivas *et al.*, 1998; Dorrance *et al.*, 2004).

The challenges associated with synthetic fungicides have led to the search for more effective and environmentally friendly alternative solutions (Wilson *et al.*, 1993). Several studies have been conducted to explore the potential of essential oils against phytopathogenic fungi (Neri *et al.*, 2006; Amiri *et al.*, 2008). Due to their natural origin, these oils contribute to the safety of humans and the environment. Furthermore, they present a low risk of development of resistance by pathogenic microorganisms (Tatsadjieu *et al.*, 2010). The purpose of this study is to investigate the antifungal properties of essential oils from four medicinal plants against post-harvest phytopathogenic fungi of potatoes.

I-Methods and Materials

I-1-Plant Material

The plants used to test the antifungal properties of their essential oils are widely cultivated medicinal plants family Lamiaceae, namely spearmint (*Mentha spicata*), peppermint (*Mentha piperita*), pennyroyal (*Menthapulegium*), and basil (*Ocimum basilicum*). Their aerial parts were harvested in August 2020, in the Ouargla region (Southeast Algeria) coordinates (N31°57'47" E 5°20'31"), and then dried in the shade for 15 days.

I-2-Fungal Material

The fungi used in this study were isolated from potato tubers showing characteristic symptoms of fungal attack, obtained during post-harvest from the El Oued province coordinates (N33°07',

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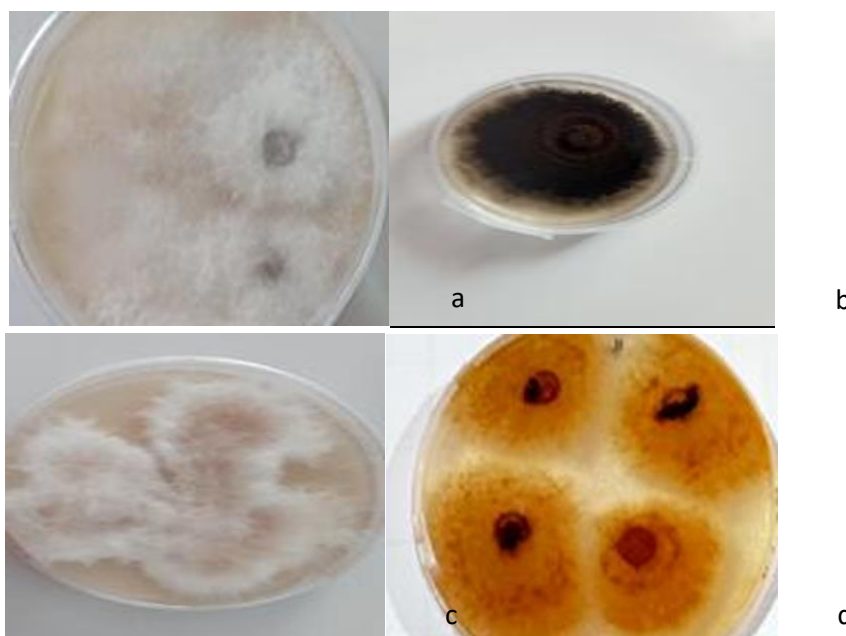


Photo 01: Phytopathogenic fungi on PDA agar.

a: *Fusarium proliferatum*, **b:** *Alternaria alternata*, **c:** *Wickerhamomyces anomalus*, **d:** *Rhizoctonia solani*.

I-3-Extraction of Essential Oils

The extraction of essential oils was performed by hydrodistillation using a Clevenger-type apparatus. Approximately 100 g of dried aerial parts of plants were immersed in a 1000 ml flask containing a sufficient volume of water and subjected to extraction for 3 hours. The resulting oil was stored in the dark at 4°C (Kizil *et al.*, 2010).

I-4-Determination of the Chemical Composition of Essential Oils by GC/MS

The chromatographic analysis of the essential oil was performed using a Bruker SCION 436 GC gas chromatography system coupled to a mass spectrometer (GC/MS). Electron impact fragmentation at 70 eV was used. The column used was an HP-5MS capillary column (15m x 0.25mm) with a film thickness of 0.25µm. The stationary phase of the column consisted of 5% phenyl and 95% dimethylpolysiloxane. The operating conditions were as follows: injector temperature of 250°C in a split mode of 1:50, temperature programming from 70°C to 280°C at a rate of 10°C/min, and the carrier gas used was helium with a flow rate of 1.5 ml/min. The temperatures of the quadrupole source were set at 250°C and 220°C, respectively. The linear retention indices (kI) for all compounds were determined using n-alkanes C10-C40. The identification of different constituents was carried out by comparing their mass spectra with those of reference products in available computerized libraries (NIST and Wiley).

I-5-Antifungal Activity

I-5-1-Direct Contact Methods

According to **Mohammedi *et al.*, 2012**, to evaluate the antifungal activity of the essential oils, we used the direct contact technique. This involved adding the oil at various concentrations to the liquid PDA culture medium at a temperature of 25°C, followed by thorough mixing for 2 minutes to ensure homogeneity. After agitation, the mixture (PDA + EO + a few drops of Tween 20) was poured into 50mm Petri dishes. Once solidified, inoculation was performed under a hood by placing a 5mm diameter mycelial disk obtained from a young culture (7 days of incubation) at the center of each Petri dish.

Control experiments were conducted under the same conditions without essential oil. The Petri dishes (both control and test samples) were incubated in a 25°C ± 2°C environmental chamber. Three replicates per treatment were performed to assess the antifungal effect of the tested oils. The radial mycelial growth was measured daily for a 10-day incubation period. The following parameters were evaluated:

I-5-2-Mycelial Growth

Mycelial growth (expressed in mm) was evaluated at the end of the experiment, after 10 days of incubation (240 hours), by measuring the average of three perpendicular diameters passing through the center of the mycelial disk. This measurement was always compared to the control cultures that started on the same day and under the same conditions.

I-5-3-Determination of Antifungal Index

For each treatment, the antifungal index, expressed as a percentage, is calculated by the reduction in mycelial diameter growth compared to the control, using the following formula:

$$I (\%) = [1 - (D_{\text{test}} / D_{\text{control}})] \times 100 \text{ (Kordali *et al.*, 2003).}$$

D_{control}: Mycelial diameter growth in a medium without the presence of the essential oil (control).

D_{test}: Mycelial diameter growth in the presence of the essential oil (test).

I-6- Statistical Analysis

Based on the obtained results for each parameter, we calculated the means, standard deviations, and conducted an analysis of variance (ANOVA) using XLSTAT software (2019).

II/ Results and Discussion

II-1- Essential Oil Extraction Yields

The essential oil extraction yields (w/w, %), obtained after a 3-hour period of hydrodistillation of the tested plant species, are presented in **table 01**.

Table 01: Essential oil yield of the plants studied

	<i>Mentha spicata</i>	<i>Mentha piperita</i>	<i>Mentha pulegium</i>	<i>Ocimum basilicum</i>
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Yields (%)	1.4	0.83	2.1	0.19
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The data analysis from the above table reveals that the essential oil yields vary among the plant species and range from 0.19 to 2.1%. *M. Pulegium* exhibited the highest essential oil content with a yield of 2.1%, followed by *M.spicata* (1.4%), *M. piperita* (0.83%), and *O. basilicum* (0.19%).

The yield of *M. spicata* is higher than the one reported by Dib *et al.* (2013) which mentioned 1.27%. Similarly, the yield of *M. piperita* is higher than the one reported by Likibi *et al.* (2015) which corresponds to 0.52%. Our results regarding *M. pulegium* (1.9%) are close to those obtained by Uwineza (2018) in Morocco. However, the yield of essential oil in *O. basilicum* is lower than 0.44% reported by Akono *et al.* (2012) and 0.63% by Kpodekon *et al.* (2013). These observed differences in yield could be attributed to the collection area, soil properties, plant developmental stage, and the organs and extraction methods used.

II-2- Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of the Essential Oil.

The chemical composition of the studied plant's essential oils was determined using gas chromatography coupled with mass spectrometry (GC-MS). The identification of the chemical composition of the essential oils (Table 02) revealed a number of compounds, representing 82.58% of the total composition of *M. spicata*, 92.19% of *M. piperita*, and 89.52% of *M. pulegium*. The total chemical composition percentage of *O.basilicum* essential oil is 86.41%.

Table 02: Chemical composition of the essential oils of the studied plants.

Compounds	kIexp	kIlit	<i>Mentha spicata</i>	<i>Mentha pepirita</i>	<i>Mentha pulegium</i>	<i>Ocimum basilicum</i>
5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	915	924	0.03	0.27	—	—
α -Pinene	930	939	0.33	1.28	—	—
Camphene	938	946	0.16	0.31	0.49	—
(-)-Sabinene	962	967	0.86	—	—	—
β -Pinene	974	979	2.8	6.43	1.32	0.42
Myrcene	981	990	—	—	0.62	0.33
exo-2,7,7-trimethylbicyclo[2.2.1]heptan-	1124	1170	10.28	18.84	—	—

Eucalyptol	1026	1031	–	–	5.78	4.72
β -Ocimene	1044	1044	0.16	0.13	–	–
3-Carene	1008	1008	–	0.11	0.02	–
Terpinolen	1181	1188	0.08	0.09	–	–
Neo-dihydrocarveol	1180	1190	–	–	0.36	–
Linalool	1085	1100	–	0.54	–	26.16
(-)-dihydrocarveol	1119	1119	–	–	0.15	–
(+)-(E)-Limonene oxide	1122	1140	0.28	0.10	–	–
Myroxide	1140	1160	–	–	–	0.17
L-Camphor	1141	1143	–	–	–	0.64
cis-Verbenol	1144	1144	–	–	0.45	–
L-Menthone	1148	1150	–	–	2.21	0.15
Isoborneol	1149	1158	5.83	2.27	0.14	0.39
cis-Dihydrocarvone	1184	1190	4.71	7.96	6.62	–
L- α -Terpineol	1186	1186	2.23	–	3.08	–
Estragole	1190	1208	–	–	–	16.69
Isopulegone	1208	1208	–	–	23.62	1.70
Fenchyl acetate	1223	1232	–	–	–	0.18
D-Carvone	1243	1239	–	–	–	0.89
Chavicol	1247	1250	–	–	–	2.38
cis-Carvotanacetol	1248	1244	–	–	0.10	–
Citronellyl formate	1250	1260	–	–	0.31	–
(-)-Carvone	1258	1243	41.66	48.74	30.89	–
Bornyl acetate	1285	1270	–	0.13	–	1.42

Geranyl formate	1298	1300	–	–	0.22	–
Dihydrocarvyl acetate	1301	1303	0.04	0.19	–	–
α –Cubebene	1342	1348	–	–	–	0.12
Piperitenone	1344	1343	–	0.33	2.58	–
Phenol, 2-methoxy-3-(2-propenyl)-	1360	1360	0.08	–	–	4.33
cis-Carvyl acetate	1362	1362	0.10	0.12	–	–
Jasmone	1370	1380	–	–	0.07	–
α -Copaene	1388	1388	–	–	–	0.34
β -Bourbonene	1388	1389	1.28	–	0.94	0.46
(-)- β -elemene	1389	1390	–	0.09	0.10	2.09
trans- α -Bergamotene	1410	1411	–	–	–	2.88
Geranylacetone	1420	1430	0.19	0.07	0.23	–
β -farnesene	1448	1442	–	–	–	0.77
Humulene	1449	1454	0.92	–	0.87	–
cis-Muurola-4(15),5-diene	1460	1466	0.43	0.20	0.23	0.74
Germacrene D	1478	1485	0.21	0.10	0.10	–
α -Bulnesene	1490	1505	–	–	–	0.99
Germacrene A	1500	1508	0.03	–	–	–
(-)-gamma-cadinene	1504	1514	0.89	–	0.31	–
Calamenene	1517	1522	0.47	0.21	0.22	0.58
Sesquisabinene	1519	1534	–	–	–	0.18
Cubebol	1530	1535	–	–	–	0.33
Nerolidol	1558	1560	–	–	–	1.02

(-)-Spathulenol	1570	1577	–	–	–	3.11
Caryophyllene oxide	1575	1583	1.70	0.54	2.40	–
(-)-Globulol	1576	1590	–	–	–	0.34
Humulene epoxide II	1599	1597	0.20	–	0.05	–
Epicubenol	1606	1612	0.98	0.38	0.84	2.15
T-Cadinol	1613	1638	4.41	2.41	3.14	8.50
α -Cadinol	1641	1652	0.72	0.27	0.48	–
4(15),5,10(14)- Germacratrien-1-ol	1670	1681	0.10	–	–	–
Caryophylladienol II	1672	1678	0.22	–	0.29	–
Bisabolol	1675	1685	0.20	0.08	0.29	0.92
β -Sinensal	1678	1699	–	–	–	0.13
Aromadendrane	1679	1678	–	–	–	0.19
Total %			82.58	92.19	89.52	86.41
Hydrocarbon monoterpenes			4.42%	8.62%	2.45%	0.75%
Oxygenated monoterpenes			65.07%	78.78%	75.98%	55.84%
Hydrocarbon sesquiterpenes			4.23%	0.6%	2.77%	8.57%
Oxygenated sesquiterpenes			8.53%	3.68%	7.49%	16.69%
Others			0.33%	0.51%	0.83%	4.56

kI exp: Experimental retention index. kI lit: Literature retention index.

These results indicate that the essential oil of *M. spicata* (Table 02) is primarily composed of monoterpenes (69.49%), with a predominance of oxygenated compounds (65.07%), while hydrocarbon monoterpenes constitute a minor fraction (4.42%). Sesquiterpenes account for 12.76% of the composition, with a prevalence of oxygenated compounds (8.53%) and hydrocarbon sesquiterpenes (4.23%). The essential oil of *M. piperita* is also predominantly monoterpenic (87.4%), with a higher proportion of oxygenated monoterpenes (78.78%) compared to hydrocarbon monoterpenes (8.62%). Sesquiterpenes represent 4.28% of the composition, with a prevalence of oxygenated compounds (3.68%) and hydrocarbon sesquiterpenes (0.6%). The essential oil of *M. pulegium* is primarily composed of monoterpenes (78.43%), with a predominance of oxygenated monoterpenes (75.98%), while hydrocarbon monoterpenes constitute a minor fraction (2.45%). Within the sesquiterpene group (10.26%), oxygenated compounds account for 7.49%, while hydrocarbon sesquiterpenes occupy a proportion of 2.77%. The essential oil of *O. basilicum* is predominantly monoterpenic (56.59%), with the majority composed of oxygenated monoterpenes (55.84%), while hydrocarbon monoterpenes are in the minority (0.75%). Sesquiterpenes represent 25.26% of the composition, with 16.69% being oxygenated compounds and 8.57% being hydrocarbon compounds.

The essential oils of *M. spicata*, *M. piperita* and *M. pulegium* are mainly composed of Carvone, with respective values of 41.66%, 48.74%, and 30.89% (Table 02). The main constituent of *O. basilicum* oil is Linalool (26.16%).

Mentha spicata is composed of (-)-Carvone (41.66%), exo-2,7,7-trimethylbicyclo[2.2.1]heptane (10.28%), isoborneol (5.83%), cis-Dihydrocarvone (4.71%), T.-Cadinol (4.41%), β -Pinene (2.8%), L- α terpineol (2.23%), caryophyllene oxide (1.70%), and β -Bourbonene (1.28%), totaling 85.41%. These results are consistent with most of the previous studies conducted. In Algeria, the essential oil of *Mentha spicata* is predominantly composed of carvone (59.40%), limonene (6.12%), and 1,8-cineole (3.80%) (Boukhubti *et al.*, 2011). In Morocco, carvone (29%) and trans-carveol (14%) were predominant (Znini *et al.*, 2011). In Tunisia, carvone (40.8%) and limonene (20.8%) were found to be major constituents (Snoussi *et al.*, 2015), while in Iran, carvone constituted 78.78% and limonene accounted for 11.50% (Shahbazi *et al.*, 2015).

Mentha piperita is primarily composed of (-)-Carvone (48.47%), accompanied by other constituents in relatively low amounts: 2,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol (18.84%), cis-Dihydrocarvone (7.96%), beta-Pinene (6.43%), T.-Cadinol (2.41%), and Isoborneol (2.27%), α -Pinene (1.28%), totaling approximately 92.77%. When comparing the chemical composition of *Mentha piperita* essential oils from different geographical origins worldwide, we observe that the oil from Algerian origin is similar to the one studied in our research.

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The essential oil of *Mentha piperita* is predominantly composed of Carvone (51.04%), β -Pinene (1.66%), Caryophyllene (0.37%), and α -Pinene (1.07%) in Algerian oil (Goudjil *et al.*, 2016), while in Brazilian essential oil, Carvone is also the major compound, but with a percentage of 30.5% (Gracindo *et al.*, 2006). However, the essential oil differs from the one from Congo, where the major constituents are Methyl acetate (36.96%), Menthol (41.81%), and L-Menthone (5.12%) (Likibi *et al.*, 2015).

Indeed, it differs from the essential oil of Serbia, which is composed of menthol (37.4%), Methyl acetate (17.4%), and menthone (12.7%) (Sokovic *et al.*, 2009), as well as the essential oil of Turkish origin, which consists of (+)-menthol (38.06%), menthol (35.64%), and neo-menthol (6.73%) (Kizil *et al.*, 2010). The essential oil from Iran contains menthofuran (11.18%) and 1,8-cineole (6.69%) (Mohammed *et al.*, 2012). The essential oil from Morocco is dominated by menthone (29.01%), followed by menthol (5.58%), and Methyl acetate (3.34%) (Derwich *et al.*, 2010). The chemical composition of the Egyptian essential oil (Gharib and Teixeira da Silva, 2012) differs from our oil due to the presence of 1,8-cineole (8.69%) as well as a high content of neo-menthol (40.47%).

The essential oil of *Mentha pulegium* is characterized by the presence of (-)-carvone as the main component, accounting for 30.89% of the oil composition. Other significant constituents include isopulegone (23.62%), cis-Dihydrocarvone (6.62%), eucalyptol (5.78%), T-Cadinol (3.14%), L- α -Terpineol (3.08%), Piperitenone (2.58%), Caryophyllene oxide (2.40%), L-Menthone (2.21%), and β -Pinene (1.32%), totaling 90.03%. These results differ from most studies conducted by various authors. For example, Hmiri *et al.* (2011) in Morocco reported that the major component was R(+)-Pulegone at 80.28%. Aissaoui *et al.* (2018) and Uwineza *et al.* (2018) showed that Pulegone was the principal component at concentrations of 67.63% and 84.75%, respectively. Additionally, studies conducted in Tunisia by Snoussi *et al.* (2008) and Hajlaoui *et al.* (2009) revealed that Pulegone was the main compound in *Mentha pulegium*, with percentages of 44.27% and 61.11%, respectively. In Algeria, the major components of the essential oil are Pulegone (38.81%), Menthone (19.24%), and Piperitenone (16.52%) (Boukhubti *et al.*, 2011).

The volatile essence of *O. basilicum* obtained in our results contains linalool (26.16%) as the major compound, followed by estragole (16.69%), T-Cadinol (8.50%), eucalyptol (4.72%), 2-methoxy-3-(2-propenyl)-4-hydroxyphenol, (-)-spathulenol (3.11%), trans- α -bergamotene (2.88%), chavicol (2.38%), epicubenol (2.15%), (-)- β -elemene (2.09%), isopulegone (1.70%), bornyl acetate (1.42%), and nerolidol (1.02%), totaling 87.38%. Our results are similar to the majority of studies, such as in Australia where linalool (28.6%), methyl chavicol (21.7), (E)-methyl cinnamate (14.3), α -cadinol (7.1), eugenol (5.9), and 1,8-cineole (4.0) were identified (Politeo *et al.*, 2007), in Bulgaria where linalool (54.95%), methyl chavicol (11.98%), and methyl cinnamate (7.24%) were found (Opalchenova, 2003), and in the USA where linalool

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Generally, the variation in the chemical composition of essential oils from the same species can be attributed to geographical origin, extraction technique, harvest time, and climatic factors.

II-3- Antifungal Activity

II-3-1- Mycelial Growth

The antifungal activity is determined by the absence or presence of mycelial growth. The analysis of variance indicates a highly significant difference ($p < 0.001$) for the mycelial growth of the tested fungal strains.

Figure 01 represents the fungal species growth results obtained using *Mentha spicata* essential oil. All fungal strains showed hyphal development at concentrations of 0.80% and 0.85%. Mycelial growth slightly decreases with increasing essential oil concentration compared to the control, with a diameter of 11.6mm for *Wickerhamomyces anomalus* at a concentration of 0.90% and 9mm for *Rhizoctonia solani*. For *Alternaria alternata*, a diameter of 5.4mm was recorded at a concentration of 0.88%, but no growth (0mm) was observed for *Fusarium proliferatum* at 0.90%. No mycelial growth was obtained at essential oil concentrations of 0.90% and 1%. The essential oils also exhibit inhibitory effects on the fungi, with minimum inhibitory concentrations (MIC) of 0.90% for *F. proliferatum* and *A. alternata*, and 0.95% for *W. anomalus* and *R. solani*.

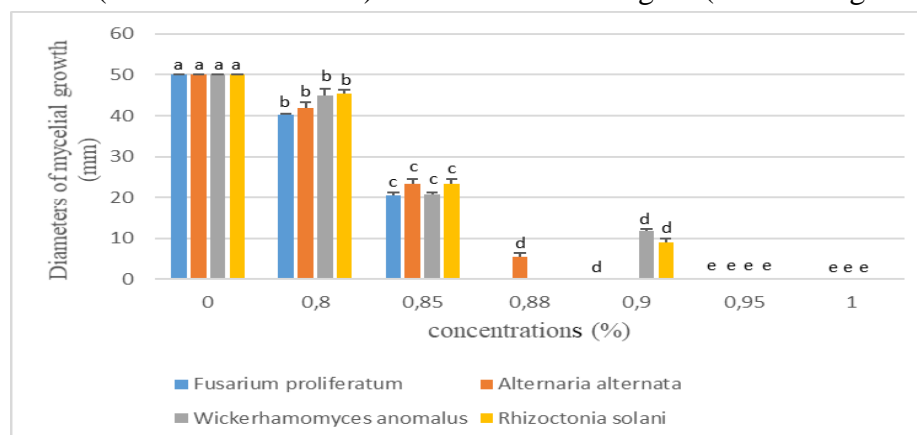


Figure 01: Effect of different concentrations of *Mentha spicata* essential oils on mycelial growth.

Figure 02 shows that *Mentha piperita* essential oil inhibits the growth of *Fusarium proliferatum*, *Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani*. No mycelial growth was observed at 0.95% and 1% concentrations. At 0.90% concentration, a mycelial growth diameter of 14.2 mm was recorded for *Fusarium proliferatum* and 10.2 mm for *Rhizoctonia solani*, but no growth (0 mm) was observed for *Alternaria alternata* and *Wickerhamomyces anomalus*. At 0.85% concentration, a mycelial growth diameter of 9 mm was noted for *Wickerhamomyces anomalus*, and 0 mm at 0.87%. For *Fusarium proliferatum*, a diameter of 34.4 mm was recorded, for *Alternaria alternata* (20.2 mm), and for *Rhizoctonia solani* (20 mm). The MIC was determined to be 0.95% for *Fusarium proliferatum* and *Rhizoctonia solani*, 0.90% for *Alternaria alternata*, and 0.87% for *Wickerhamomyces anomalus*. The largest mycelial growth diameter (50 mm) was recorded in the control.

The analysis of variance indicates a highly significant difference ($p < 0.001$) for the mycelial growth of the tested fungal strains.

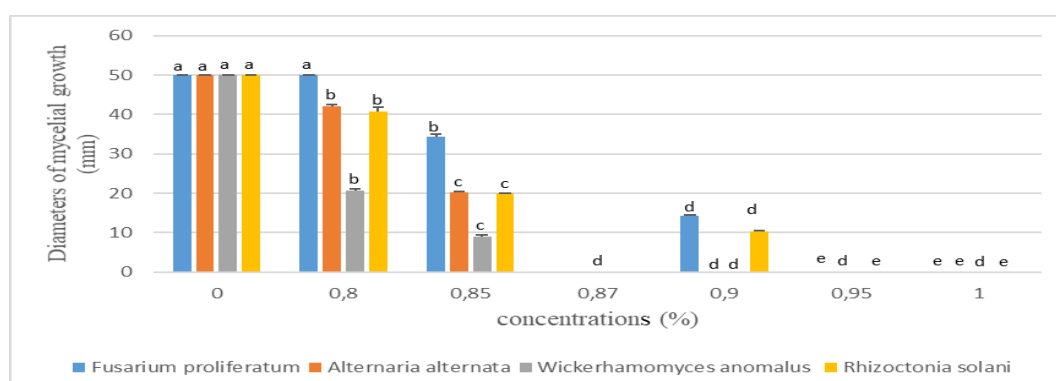


Figure 02: Effect of different concentrations of *Mentha piperita* essential oils on mycelial growth.

Antifungal activity of essential oils of four medicinal plants on fungi isolated from potato tubers (*Solanum tuberosum*) from the El Oued region (Eastern Algerian Northern Sahara)

Figure 03 shows the results of the effects of *Mentha pulegium* on the growth of fungal species.

The analysis of variance reveals a highly significant difference ($p < 0.001$) for the mycelial growth of the tested fungal strains.

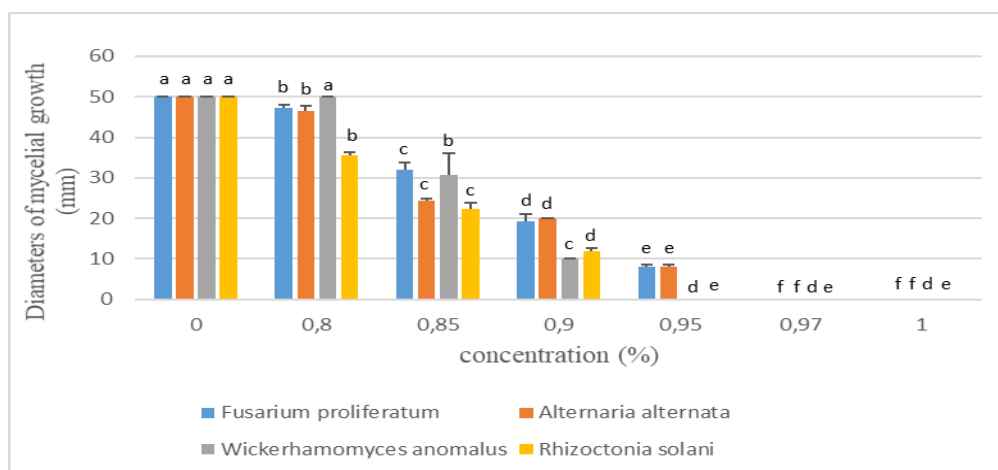


Figure 03: Effect of different concentrations of *Mentha pulegium* essential oils on mycelial growth.

A maximum mycelial growth diameter (50 mm) of *Fusarium proliferatum*, *Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani* was obtained in the control group, corresponding to the absence of essential oils. Mycelial growth was observed for all strains at concentrations of 0.80%, 0.85%, 0.90%, and 0.95% in the presence of the essential oil of *Mentha pulegium*, except for *Wickerhamomyces anomalus* and *Rhizoctonia solani* at a concentration of 0.95% (0 mm). Mycelial growth slightly decreased with increasing essential oil concentration compared to the control. At concentrations of 0.97% and 1%, a diameter of 0 mm was recorded for all strains. The minimum inhibitory concentration (MIC) was determined to be 0.97% for *Fusarium proliferatum* and *Alternaria alternata*, and 0.95% for *Wickerhamomyces anomalus* and *Rhizoctonia solani*.

The analysis of variance indicates a highly significant difference ($p < 0.001$) for the mycelial growth of the tested fungal strains. According to Figure 04, the effect of *Ocimum basilicum* essential oil was only observed at concentrations of 0.97% and 1%, where no growth was obtained for all the fungi. Mycelial growth slightly decreased with increasing essential oil concentration compared to the control, with a diameter of 4.2 mm for *Fusarium proliferatum*, 8.2 mm for *Alternaria alternata*, and 10 mm for *Wickerhamomyces anomalus* at a concentration of 0.95%. However, for *Rhizoctonia solani*, it reached 4 mm. In the control group, all four strains exhibited growth, reaching a diameter of 50 mm. The minimum inhibitory concentration (MIC) was determined to be 0.97% for *Fusarium proliferatum*, *Alternaria alternata*, and *Rhizoctonia solani*, and 1% for *Wickerhamomyces anomalus*.

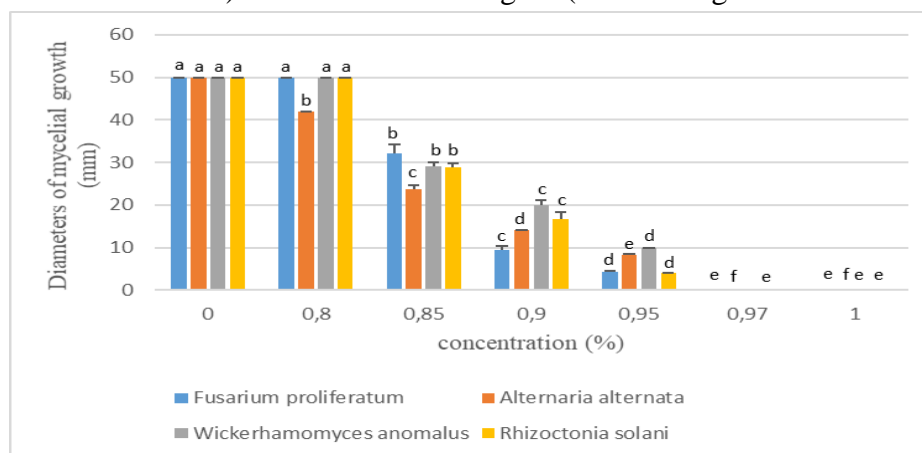


Figure 4: Effect of different concentrations of *Ocimum basilicum* essential oil on mycelial growth.

II-3-2. Determination of antifungal index

Regarding *Mentha spicata*, Figure 5 also shows 100% inhibition of mycelial growth for *Fusarium proliferatum*, *Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani* at concentrations of 1% and 0.95%, as well as at 0.90% for *Fusarium proliferatum*. We obtained 19.6% inhibition at the concentration of 0.80% for *Fusarium proliferatum*, 16.4% for *Alternaria alternata*, 10% for *Wickerhamomyces anomalus*, and 9.2% for *Rhizoctonia solani*. The antifungal index increases as the concentration of the essential oil increases, reaching 89% at the concentration of 0.88% for *Alternaria alternata*.

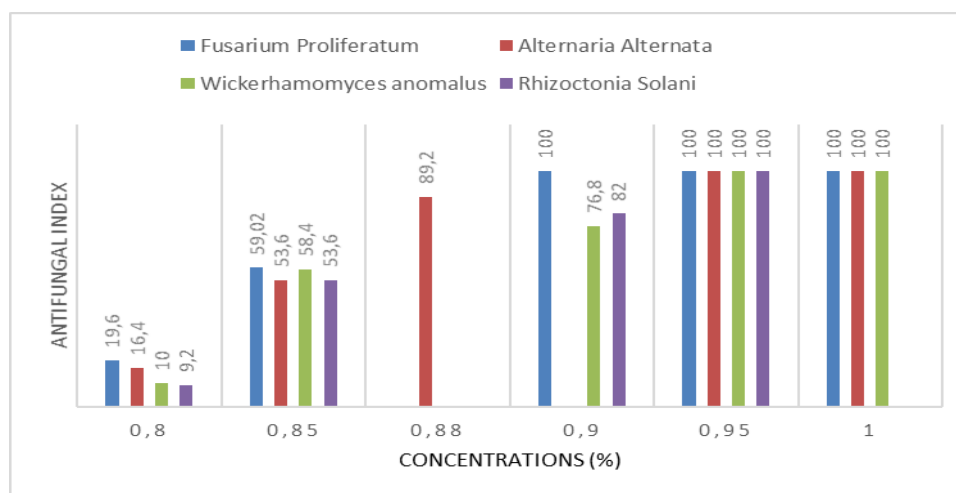


Figure 5: Antifungal index of inhibition of mycelial growth for different concentrations of *Mentha spicata* essential oil.

Figure 6 also shows a complete inhibition (100%) of mycelial growth for all tested strains at concentrations of 1% and 0.95%. At 0.90%, effectiveness is observed for *Fusarium proliferatum*, *Alternaria alternata*, and *Wickerhamomyces anomalus*, and at 0.87% for *Wickerhamomyces*

Antifungal activity of essential oils of four medicinal plants on fungi isolated from potato tubers (*Solanum tuberosum*) from the El Oued region (Eastern Algerian Northern Sahara) *anomalus*. However, we obtained 0% inhibition at the concentration of 0.80% for *Fusarium proliferatum*, 15.6% for *Alternaria alternata*, 58.8% for *Wickerhamomyces anomalus*, and 18.4% for *Rhizoctonia solani* of essential oil. The antifungal index increases as the concentration of the essential oil is increased, reaching 0.85%.

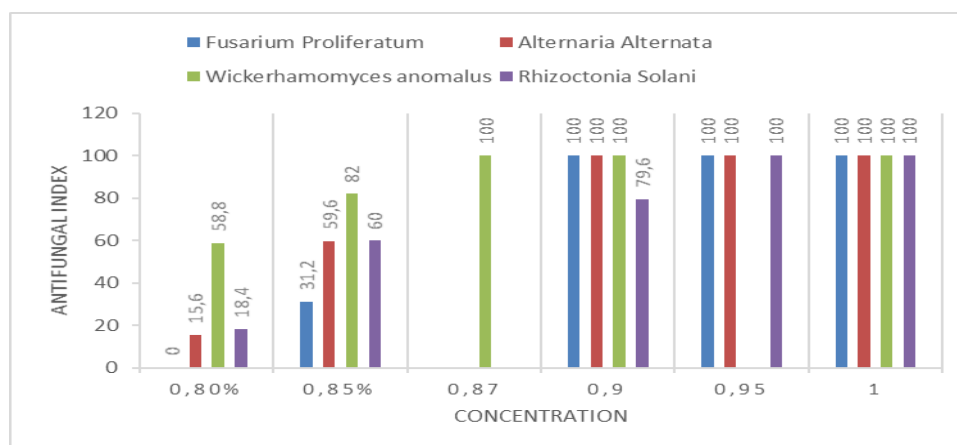


Figure 6: Antifungal index of inhibition of mycelial growth for different concentrations of *Mentha piperita* essential oil.

Mentha pulegium essential oil strongly inhibits the growth of strains (Figure 7). A 100% fungicidal inhibitory effect was observed at concentrations of 1% and 0.97% for all strains, and at 0.95% for *Wickerhamomyces anomalus* and *Rhizoctonia solani*. Effectiveness was recorded for *Fusarium proliferatum* and *Alternaria alternata* strains with an antifungal index of 83.6%. However, at concentrations of 0.80%, 0.85%, and 0.90%, the antifungal index increases as the concentration of the essential oil increases.

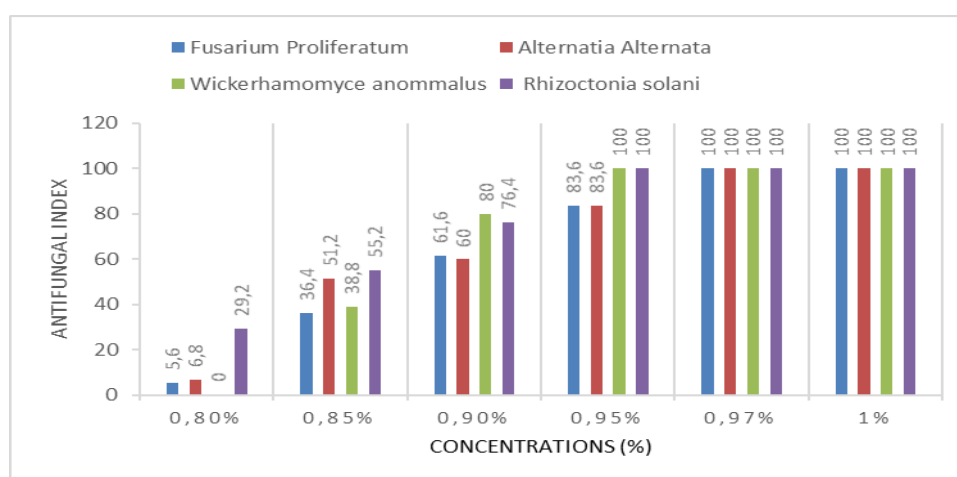


Figure 7: Antifungal index of inhibition of mycelial growth for different concentrations of *Mentha pulegium* essential oil.

On Figure 8, we observe a complete inhibition (100%) of mycelial growth for the fungal strains at concentrations of 0.97% and 1%. However, we obtained 16% inhibition at the

Antifungal activity of essential oils of four medicinal plants on fungi isolated from potato tubers (*Solanum tuberosum*) from the El Oued region (Eastern Algerian Northern Sahara) concentration of 0.80% for *Alternaria alternata* and no inhibition (0%) for *Fusarium proliferatum*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani*. At the concentration of 0.90%, we recorded 81.2% inhibition for *Fusarium proliferatum*, 72% for *Alternaria alternata*, 60.4% for *Wickerhamomyces anomalus*, and 66.8% for *Rhizoctonia solani*.

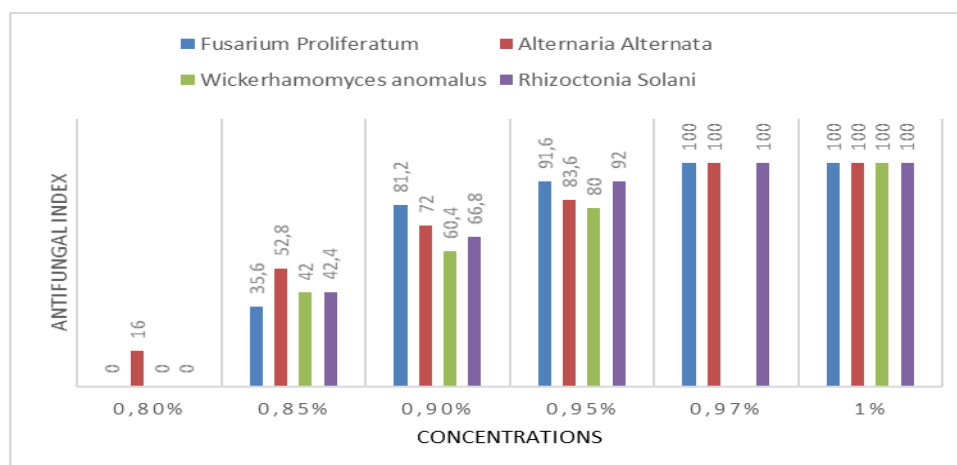


Figure 8: Antifungal index of inhibition of mycelial growth for different concentrations of *Ocimum basilicum* essential oil.

The effect of essential oils from *Mentha spicata*, *Mentha piperita*, *Mentha pulegium*, and *Ocimum basilicum* on the development of these phytopathogenic fungi varies according to the concentration. The lowest concentration completely inhibited the hyphal growth of the tested strains. This activity is likely due to the nature and molecular structure of the active ingredients in the essential oil. These compounds penetrate the cell membrane, enter the cell, interact with key intracellular sites such as enzymes and proteins, and induce cell death (Omidygi *et al.*, 2007).

The antifungal activity of the tested plant essential oils was found to be effective at all concentrations against the fungal strains. Thus, the inhibition rate increases with the decrease in hyphal growth until complete inhibition of the disc. Our results demonstrate some variability among the fungal strains in response to the essential oils. Specifically, *Alternaria alternata* and *Wickerhamomyces anomalus* are more sensitive than *Fusarium proliferatum* and *Rhizoctonia solani*. The latter two strains likely exhibit inherent cellular resistance, and the difficulty in developing antifungal molecules has been identified as being related to the fungal cell's ultrastructure, which poses three barriers - the chitin cell walls, membrane ergosterol, and the eukaryotic nucleus (Chami, 2005), as well as the antifungal molecules themselves, which can generate resistance (Prasad and Kapoor, 2005).

The essential oil of *Mentha spicata* (spearmint) exhibits potent antifungal activity against the tested strains. This is supported by a study conducted by Ismaili *et al.* (2014), which demonstrated significant inhibition of dermatophyte growth. Similarly, in our study, the

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Antifungal activity of essential oils of four medicinal plants on fungi isolated from potato tubers (*Solanum tuberosum*) from the El Oued region (Eastern Algerian Northern Sahara) essential oil of *Mentha piperita* (peppermint) shows strong antifungal activity against the tested strains. This is consistent with the findings of Goujil *et al.* (2016), who revealed that carvone, the main ingredient in peppermint essential oil, is considered responsible for its antifungal property. Furthermore, Ferdes *et al.* (2012) mentioned that essential oils from aromatic plants such as lemon and mint possess antifungal activity against other fungi such as *Aspergillus niger*, *Fusarium oxysporum*, *Monascus purpureus*, and *Penicillium hirsutum*. Additionally, peppermint oil was found to be the most effective against all the studied fungi.

The essential oil of *M. pulegium* (pennyroyal) also had an effect on the tested strains. Lahlou *et al.* (2005) demonstrated that the essential oil of this plant inhibited the growth of *Penicillium spp.* Similarly, Hajlaoui *et al.* (2009) reported that the pennyroyal oil from Tunisia, which is rich in menthone (61.11%), caused inhibition of cultures of *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, and *Trichoderma sp.* Smid *et al.* (1995) and Hmiri *et al.* (2013) studied the inhibitory effect of fifteen constituents of *M. pulegium* essential oil on the germination of *Penicillium hirsutum* conidia. Their results showed that pulegone, menthone, menthol, and carvone completely inhibited conidial germination, with carvone exhibiting the strongest inhibitory effect.

The essential oil of basil (*Ocimum basilicum*) induced complete inhibition of mycelial growth in the tested fungal strains. Edris and Farrag (2003) and Doumouya *et al.* (2021) found that the vapor of basil essential oil and its main compound, linalool, inhibited the growth of *Mucor sp.* and *Rhizopus stolonifer* in a dose-dependent manner. Additionally, Awuah and Ellis (2002) and Doumouya *et al.* (2021) demonstrated the effectiveness of basil leaf powder in protecting peanut stocks against *Aspergillus parasiticus* and stock contaminants.

The different observed inhibition rates suggest that the various essential oils have interesting antifungal effects against fungal strains associated with potato tuber rot. In general, the fungal strains showed sensitivity to increasing dosages, resulting in a progressive increase in the percentage of inhibition. Therefore, this inhibitory activity is "dose-dependent." The effectiveness of these oils could be attributed to their antifungal properties, which allow them to halt or slow down fungal mycelial production. According to Kalemba and Kunicka (2003) and Doumouya *et al.* (2021), the components of essential oils, such as terpenes and phenylpropanols, confer antibacterial and antifungal properties. Furthermore, the antifungal activity of essential oils can be attributed to the synergistic effect among their different compounds, with the major compounds often being responsible for the antifungal activity. However, minor components may also contribute significantly to the activity of essential oils.

Conclusion

The study focused on the antifungal activity of essential oils from four aromatic and medicinal plants cultivated in Algeria against four phytopathogenic fungi responsible for post-harvest

Antifungal activity of essential oils of four medicinal plants on fungi isolated from potato tubers (*Solanum tuberosum*) from the El Oued region (Eastern Algerian Northern Sahara) deterioration of potatoes. The analysis of these essential oils obtained by hydrodistillation revealed that peppermint (*Mentha piperita*), pennyroyal (*Mentha pulegium*), and spearmint (*Mentha spicata*) mainly contain carvone, while basil (*Ocimum basilicum*) is primarily composed of linalool. The tested essential oils clearly exhibited anti-growth effects on the studied fungi. They can be used as alternatives to synthetic fungicides in strategies for controlling pathogenic agents responsible for potato tuber rot while protecting the environment. These future bio-fungicides offer a promising approach in the fight against various fungal diseases.

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