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Study of the Antifungal Activity of Aqueous Extracts from Four Medicinal Plants on Phytopathogenic Fungi Isolated from Potato (*Solanum teberosum*) in the El Oued Region (Eastern Northern Sahara - Algeria)

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Abstract

In this study, our objective was to analyze the chemical composition of aqueous extracts from the aerial parts of four plants: *Mentha spicata* L., *Mentha piperita* L., *Mentha pulegium* L., and *Ocimum basilicum* L., and to evaluate their antifungal activity against certain phytopathogenic fungi, specifically *Alternaria alternata*, *Wickerhamomyces anomalus*, *Fusarium proliferatum*, and *Rhizoctonia solani*, which were isolated from post-harvest potato tubers from the El Oued region (northern Sahara, eastern Algeria).

Maceration with agitation for 24 hours was employed to obtain the aqueous extracts. The results revealed that the yield varied between 0.13% and 0.27%. Phytochemical tests conducted on the aqueous extracts highlighted the presence of various chemical compounds such as tannins, flavonoids, saponins, and alkaloids. The content of total phenolics of different plants extracts was in the range of 3.277 - 11.124 mg. while the content of total flavonoids of the different plants extracts was in the range of 0.896 - 2.312 mg. HPLC analysis revealed that the aqueous extract of *M. piperita* is composed of acetylsalicylic acid, chlorogenic acid, and p-coumaric acid, whereas *M. pulegium* is characterized by the presence of acetylsalicylic acid, caffeine, and pyrogallol. *O. basilicum* contains gallic acid.

The aqueous extracts were tested for their antifungal activity using the direct contact method against the mycelial growth of the tested strains. The results revealed a strong antifungal potential against these strains. At concentrations of 1.5%, 2%, 3.5%, and 4%, the aqueous extract of *Mentha spicata* exhibited a 100% antifungal effect on all four fungi. Regarding *Mentha piperita*, the aqueous extract at 1% caused total inhibition (100%) of *Alternaria alternata* and *Wickerhamomyces anomalus*, and at 4% for *Fusarium proliferatum* and *Rhizoctonia solani*. We observed a fungicidal inhibitory effect (100%) at 1.5% for *Alternaria alternata* and *Wickerhamomyces anomalus*, and at 3.5% for *Fusarium proliferatum* and *Rhizoctonia solani* with the aqueous extract of *Mentha pulegium*. The aqueous extract of *Ocimum basilicum* resulted in total inhibition (100%) at concentrations of 2%, 2.5%, 5.5%, and 7.5% for *Wickerhamomyces anomalus*, *Alternaria alternata*, *Fusarium proliferatum*, and *Rhizoctonia solani* respectively.

The results obtained pave the way for the exploitation of the aerial parts of the investigated plants as an alternative to synthetic chemical fungicides.

Keywords: Potato, medicinal plants, phytopathogenic fungi, aqueous extracts, antifungal activity.

1. Introduction

The potato (*Solanum tuberosum* L.), belonging to the Solanaceae family, is the fourth most important staple food in the world, following wheat, rice, and maize. According to the United Nations Food and Agriculture Organization (FAO), this crop covers more than 17 million hectares worldwide, with global production exceeding 370 million tons **(FAOSTAT, 2021).**

The significance of the potato among staple crops lies in its highly nutritious properties, its potential for both industrial and domestic use, and its accessibility to consumers from low-income backgrounds **(Zaheer and Akhtar, 2016).**

Each year, a considerable portion of global agricultural production is lost due to various pests and diseases, which have a significant impact on agricultural yields **(Whish et al., 2014).** The majority of these diseases are caused by harmful fungi, accounting for approximately 20 to 40% of losses **(McDonald and Stukenbrock, 2016)**.

To minimize post-harvest losses, perishable products are treated with synthetic chemical fungicides. However, some of these fungicides are carcinogenic, highly toxic, have long degradation periods, and cause environmental pollution. They pose a threat to human safety, thereby necessitating a growing shift towards agriculture free from chemical residues (**Kamele et al., 2019).**

Furthermore, chemical fungicides can lead to severe medical complications, including various types of cancer. Thus, it is essential to explore natural antifungal substances as alternative methods to combat plant diseases **(Da et al., 2019).**

Among natural resources, plant extracts from medicinal and aromatic plants may contain a variety of biologically active molecules that act directly on microbial pathology. These extracts offer advantages (medical or phytosanitary) that the commonly used fungicides often lack **(Senhaji et al., 2005; Mahesh and Satish, 2008).**

In this context, we aimed to evaluate the antifungal activity of certain extracts from medicinal and aromatic plants cultivated in southern Algeria against phytopathogenic fungi affecting potatoes.

2. Materials and Methods

2.1. Plant Material

The plants used in this study belong to the Lamiaceae family, consisting of spearmint (*Mentha spicata* L.), peppermint (*Mentha piperita* L.), pennyroyal (*Mentha pulegium* L.), and basil (*Ocimum basilicum* L.). In August 2020, the peak production period for these plants, the aerial parts were harvested in the region of Ouargla (southeastern Algeria), at the coordinates (N31°57'47" E5°20'31").

2.2.Fungal Material

In this study, four phytopathogenic fungal strains were isolated from potato tubers originating from the El Oued region (N33°07', E7°11'). The fungal species used were *Fusarium*

proliferatum, *Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani*. These strains were purified and identified by PCR, with GenBank accession numbers NCBI (National Center for Biotechnology Information) OQ606246.1, OQ860003.1, OQ771178.1, and OQ606247.1 **(Benhaoued et al., 2024).**

2.3. Preparation of Aqueous Plant Extracts

The aqueous extracts were prepared by macerating 10 g of plant powder in 100 ml of distilled water, with agitation at 200 rpm for 24 hours at a temperature of 25°C **(Razak et al., 2009; Beddou et al., 2015).** The mixture was then filtered using Whatman filter paper and centrifuged at 600 rpm for 15 minutes. The recovered filtrate was evaporated to dryness under reduced pressure at 40°C using a rotary evaporator. The yield (in %) was determined using the following formula **(Majhenic, 2007)**

$$\mathbf{Rd\ \% = (m_1\ X\ 100)\ /\ m_0}$$

Where:

- Rd: Yield

- (m_1): Mass of the dry extract (in g)

- (m_0): Mass of the dry plant material (in g)

2.4. Phytochemical Tests

A phytochemical study involves characterizing the different categories of molecules present in a plant **(Bruneton, 1999)**. To achieve this, the various obtained extracts were subjected to phytochemical tests. These tests are based on color reactions and precipitation **(Bruneton, 1999; Mojab et al., 2003; Karumi et al., 2004; Oloyede, 2005; Koffi et al., 2009).**

2.5. Quantitative Analysis

2.5.1. Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent was used to measure the total phenolic content (TPC) of the different extracts (**Singleton and Rossi, 1965**). Gallic acid (0.03-0.25 g/L) was used as a standard reference to construct the calibration curve. 0.1 ml of each extract was mixed with 0.5 ml of 10% Folin-Ciocalteu reagent. After 2 minutes, mixture was neutralized with 2 ml of 20% Na_2CO_3 solution. The reaction mixture was kept in the dark at 25°C for 30 minutes. The absorbance of the blue color was measured using a UV/Vis spectrophotometer at a fixed wavelength of 760 nm. TPC was calculated using the linear regression equation obtained from the gallic acid standard curve. The total phenolic content was calculated as the mean ± SD (n = 3) and expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (DW).

2.5.2. Determination of Total Flavonoid Content (TFC)

The total flavonoid content was estimated according to a procedure described in **(Belguidoum et al., 2015)** protocol. 1.5 ml of an ethanolic solution of $AlCl_3$ (2%) was added to 1.5ml of each extract. After 30 minutes at room temperature, the absorbance was measured

at 430 nm. The total flavonoid content was calculated as the mean ± SD (n = 3) and expressed as mg of quercetin equivalents (QE) per gram of dry weight (DW).

2.6. Characterization of Polyphenols in Aqueous Extracts

The qualitative analysis of phenolic compounds in the aqueous extracts was performed using high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) under the conditions listed in (**Table 1)**.

Table 1: Conditions for High-Performance Liquid Chromatography (HPLC) Coupled with Mass Spectrometry (MS).

Time	65.00 min
Volume injection	10 ul
Max Press	40.0 MPa
Press Min	0.0 MPa
Maximum Temperature	90° C
Mobile phase	A (ultra-pure water) B (Ethanol)
Processed by	HPLC (SHUMADZU)
Column Name	Ultra C18
Column ID	250 x 4.6 mm
Particle Size	5 A

2.7. Antifungal Activity

2.7.1 Direct Contact Methods

We employed the direct contact method **(Mohammedi et al., 2012)** to evaluate the antifungal efficacy of the aqueous plant extracts.

2.7.1.1. Mycelial Growth

Mycelial growth was measured (in mm) at the end of the experiment, after 10 days of incubation (240 hours). Measurements were taken from the average of three perpendicular diameters. These readings were always compared to control cultures that started on the same day and under the same conditions.

2.7.1.2. Determination of the Antifungal Index

According to **Kordali et al. (2003),** the antifungal index for each aqueous extract is calculated by the reduction in the fungal colony diameter relative to the control parameter, using the following formula:

$$I\ (\%) = [1 - (D_{test} / D_{Control})] \times 100$$

Where:

$D_{control}$: Mycelial diameter growth in a medium without the presence of the aqueous extracts (in mm).

D_{test}: Mycelial diameter growth in the presence of the aqueous extracts (in mm).

2.8. Statistical Analysis

The means, standard deviations, and analysis of variance (ANOVA) were calculated from the obtained results for each parameter using XLSTAT software (2019).

3. Results and Discussion

3.1. Extraction and Yields

The yields of the aqueous extracts obtained after maceration and evaporation of the 4 tested plants are presented in (**Table 2).**

Table 2: Yields of Aqueous Extracts from the Studied Plants

Space	*Mentha spicata*	*Mentha piperita*	*Mentha pulegium*	*Ocimum basilicum*
Yields (%)	0.27% ±0.01	0.13% ±0.01	0.21% ±0.01	0.17% ±0

The data analysis from(**Table 2)** reveals that the yields of the aqueous extracts vary according to the plant species and range between 0.13% and 0.27%. The yield of *Mentha spicata* is 0.27%, which is lower than that reported by **Zekri et al. (2021)** who recorded 4.9%. **Abkhoo and Jahani (2017)** indicated that the yields of plant extracts vary depending on the extraction solvent and the plant extract used. Their results showed that the yields of aqueous extracts of *Mentha spicata* were 23.8%. **Kaddour et al. (2022)** mentioned respective yields of aqueous extract of *Mentha spicata* from the regions of El-Oued, Tibessa, and El-Tarf as 13.4%, 12.7%, and 11%. The yield of *Mentha piperita* (0.13%) is lower than that reported by **Dorman et al. (2009)** who obtained 1.29%. The yield of aqueous extracts of *Mentha pulegium* (0.21%) is lower than that mentioned by **Zekri et al. (2021)** who recorded 3.8% for this species. **Khennouf et al. (2013)** obtained a yield of 14.4% from the methanolic extract of *Mentha pulegium*. The yield of *Ocimum basilicum* is 0.17%.

3.2. Phytochemical Screening

The results of the phytochemical characterization tests of the aqueous extracts of *Mentha spicata*, *Mentha piperita*, *Mentha pulegium*, and *Ocimum basilicum* are presented in (**Table 3)**. These results provide a qualitative insight into the presence or absence of bioactive molecules found in the four aqueous extracts.

Table 3: Chemical screening of plants extracts

Compounds	*Mentha spicata*	*Mentha piperita*	*Mentha pulegium*	*Ocimum basilicum*
Tannins	+	+	+	+
Anthocyanins	-	-	-	-
Anthroquinones	+	+	-	-
Saponnosides	+	+	+	+
Steroides	-	-	-	-
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+

- : Absence + : Presence

The extracts of the studied plants are rich in secondary metabolites including alkaloids, tannins, saponins, and anthraquinones. The aqueous extract of *Mentha spicata* is rich in flavonoids, saponins, alkaloids, tannins, and anthraquinones. **EL-Haoud et al. (2018)** indicated that the aqueous extract of *Mentha spicata* is very rich in polyphenols with a significant presence of cardiac glycosides. Sterols, terpenes, and flavonoids are moderately present in the aqueous extract, while reducing compounds and alkaloids were not determined. **Ullah et al. (2011)** reported that spearmint from four regions of Pakistan contains tannins, alkaloids, flavonoids, coumarins, sterols, and triterpenes. However, saponins and anthraquinones were not detected. Similarly, the study by **Naidu et al. (2012)** revealed the presence of alkaloids, flavonoids, and glucosides in the crude extract of this species. Conversely, the study by **Prasad et al. (2010)** mentioned the absence of phenols, saponins, flavonoids, cardiac glycosides, and terpenes.

The aqueous extract of *Mentha piperita* is rich in secondary metabolites such as alkaloids, saponins, flavonoids, and tannins. **Khamis and Aly (2017)** detected the presence of glycosides, flavonoids, and tannins in peppermint extract, while alkaloids, saponins, phenols, steroids, and proteins were not detected **Patil et al. (2016)** indicated that the aqueous extract of *Mentha piperita* contains diterpenes, steroids, tannins, flavonoids, carbohydrates, alkaloids, phenols, coumarin, and saponins, except for anthocyanins. **Muhammad et al. (2019)** mentioned the

presence of flavonoids, terpenoids, saponins, and phenols in the aqueous extract, except for glycosides, steroids, alkaloids, and tannins.

The phytochemical screening of the aqueous extract of *Mentha pulegium* also shows richness in secondary metabolites (alkaloids, flavonoids, saponins, and tannins). According to the study by **Rao and Tiwari (2021)**, the phytochemical screening of the extract of *M. pulegium* from the stem, leaves, and inflorescences revealed the presence of alkaloids, flavonoids, steroids, phenols, terpenoids, and tannins.

As for the extract of *Ocimum basilicum*, we detected the presence of tannins, saponins, and alkaloids. **Khamis and Aly (2017)** mentioned the presence of glycosides, phenols, flavonoids, alkaloids, saponins, steroids, proteins, tannins, and terpenoid carbohydrates. According to **Jacob et al. (2016)**, the phytochemical test of aqueous extracts of *O. basilicum* showed the presence of alkaloids, saponins, flavonoids, tannins, glycosides, steroids, and terpenoids.

3.3. Total phenolic content (TPC) and Total Flavonoid Content (TFC)

Table 4 : Total phenolic content (TPC) and Total Flavonoid Content (TFC) of plant extracts

Sample	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Mentha spicata	3.277±0.492	1.435±0.011
Mentha piperita	7,646±1,401	2.312±0.318
Mentha pulegium	5,447±0.965	0.896±0.075
Ocimum Bacilicum	11,124±1,265	1.824±0.133

The TPC of extracts varied from 3.277 to 11.124 mg of gallic acid equivalents (GAE)/g of sample DW **(Table 4)**. The highest TPC was obtained in the *Ocimum basilicum* extract, while the lowest TPC was found in the *Mentha spicata* extract. The order of TPC is: *Mentha spicata* < *Mentha pulegium* < *Mentha piperita* < *Ocimum basilicum* (**Table 4).**This study showed that the extracts have a low phenolic content compared to other studies, such as those of **Bahman et al., 2008** 150.91–433.60 mg GAE/g DW, **Politeo et al 2018**. 124.27 mg GAE/g DW, and **Karra-Bouraoui et al., 2009**. 20.1–64.5 mg GAE/g DW. D. **Gajula et al., 2009**. reported 31.37–60.47 mg GAE/g DW for *Ocimum basilicum*.

The TFC of extracts varied from 0.896 to 2.312 mg of quercetin equivalents (QE)/g of sample DW **(Table 04).** The highest TFC was obtained in the *Mentha piperita* extract, while the lowest TFC was found in the *Mentha pulegium* extract. The order of TFC is: *Mentha pulegium* < *Mentha spicata* < *Ocimum basilicum* < *Mentha piperita* (**Table 4**). This study showed that the extracts have a low flavonoid content compared to other studies, such as those of **Olivera Politeo et al**., **2018**. 12.70 mg QE/100 g DW and **Karra-Bouraoui et al., 2009**. 12.9–53.3 mg catechin E/g DW. D. **Gajula et al., 2009**. reported 4.64–6.21 mg catechin E/g DW for *Ocimum basilicum.*

3.4. HPLC Chromatography

The aqueous extract of *Mentha spicata* yielded no results when compared with the standard. According to the work of **Cirlini et al. (2016)** on the phenolic composition of *Mentha spicata* extract, HPLC analysis revealed that the extract is composed of rosmarinic acid and its derivatives with smaller quantities of salvianolic acids, caffeoylquinic acids, hydroxybenzoic acids, hydroxycinnamic acids, flavones, and flavanones.

The aqueous extract of *Mentha piperita* contains the following compounds: acetylsalicylic acid, chlorogenic acid, *para*-coumaric acid, and vitamin B1. **Dorman et al. (2009)** mention that the polyphenolic composition of aqueous extract of *Mentha piperita* contains three main polyphenols, among which are eriocitrin, luteolin-7-O-glucoside, and rosmarinic acid.

The aqueous extract of *Mentha pulegium* is composed of acetylsalicylic acid, caffeine, and pyrogallol. **Alharbi et al. (2021)** indicate the presence of seven polyphenolic compounds in the methanolic extract of *M.pulegium* such as eriocitrin, hesperidin, narirutin, luteolin, isorhoifolin, rosmarinic acid, and caffeic acid.

The aqueous extract of *Ocimum basilicum* is composed of gallic acid, while **Kanmaz et al. (2023)** detected the presence of 24 phenolic compounds. Among the main compounds found were t-caffeic acid, caftaric acid, kaempferol-3-glucoside, and quercetin-3-glucoside.

Our study conducted on the aqueous extracts of *Mentha spicata*, *Mentha piperita*, *Mentha pulegium*, and *Ocimum basilicum* revealed partially similar results to other works, namely the presence of certain chemical families, but also the absence of other chemical compounds. This can be explained by differences in several parameters, which can be geographical, physicochemical, or biological, such as the collection site, including the plant's environment, light, precipitation, topography, season, soil type, harvesting period, genetic heritage, extraction procedure used, and the plant part studied **(Malik et al., 2012; Sujana et al., 2013; Akhtar et al., 2018).**

3.5. Mycelial Growth

The antifungal activity is revealed by the absence or presence of mycelial growth in fungal strains.

3.5.1.*Mentha spicata*

The analysis of variance indicates a highly significant difference ($p<0.0001$) for the mycelial growth of the tested fungal strains.

In **Figure 1**, we can observe the impact of the aqueous extract of *Mentha spicata* on the mycelial growth of tested fungi at different doses. A maximum development (50mm) of hyphae was observed in all controls. The same applies to *Fusarium proliferatum* and *Rhizoctonia solani* at concentrations of 0.90%, 0.95%, 1%, and 1.5% for *Rhizoctonia solani*. However, with increasing concentrations, mycelial growth decreases compared to the control, eventually leading to complete inhibition. *Alternaria alternata* and *Wickerhamomyces anomalus* show a decrease in growth starting from the concentration of 0.90%, with respective diameters of

35.5mm and 41mm, reaching complete inhibition of their growth at concentrations of 1.5% for *Alternaria alternata* and 02% for *Wickerhamomyces anomalus*. No mycelial growth was obtained at a concentration of 4% for all strains. The Minimum Inhibitory Concentrations (MIC) are 3.5% for *Fusarium proliferatum*, 1.5% for *Alternaria alternata*, 2% for *Wickerhamomyces anomalus*, and 4% for *Rhizoctonia solani*.

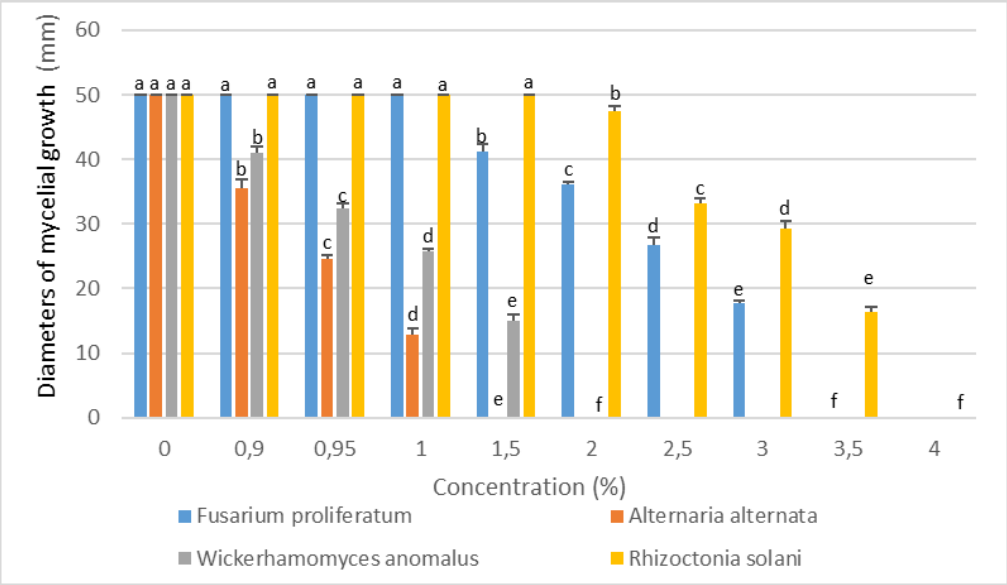

Figure 1: Effect of different concentrations of aqueous extracts of *Mentha spicata* on mycelial growth.

3.5.2.*Mentha piperita*

The analysis of variance indicates a highly significant difference ($p<0.0001$) for the mycelial growth of the tested fungal strains.

The results in **Figure 2** show a maximum diameter (50mm) of mycelial growth for the 4 fungal strains in the controls, corresponding to the absence of the aqueous extract as well as concentrations of 0.80% and 0.85%, except for *Wickerhamomyces anomalus*, which decreases in diameter to 47.5mm. The same pattern is observed at concentrations ranging from 0.90% to 1.5% for *Fusarium proliferatum* and *Rhizoctonia solani*. The mycelial growth of the fungi slightly decreases as the concentration of the aqueous extract increases, eventually leading to growth inhibition. The Minimum Inhibitory Concentrations (MIC) for *Fusarium proliferatum* and *Rhizoctonia solani* are 4%, while *Alternaria alternata* and *Wickerhamomyces anomalus* have a (MIC) of 1%.

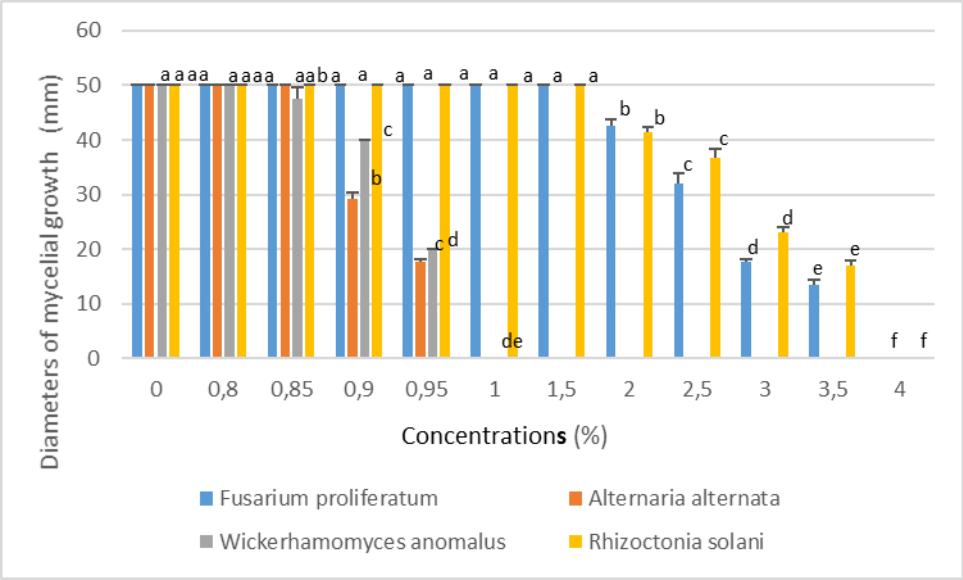

Figure 2: Effect of different concentrations of aqueous extracts of *Mentha piperita* on mycelial growth.

3.5.3.*Mentha pulegium*

Figure 3 presents the results of the effects of the aqueous extract of *Mentha pulegium* on the growth of tested fungal species. The analysis of variance indicates a highly significant difference ($p<0.0001$) for the mycelial growth of the tested fungal strains.

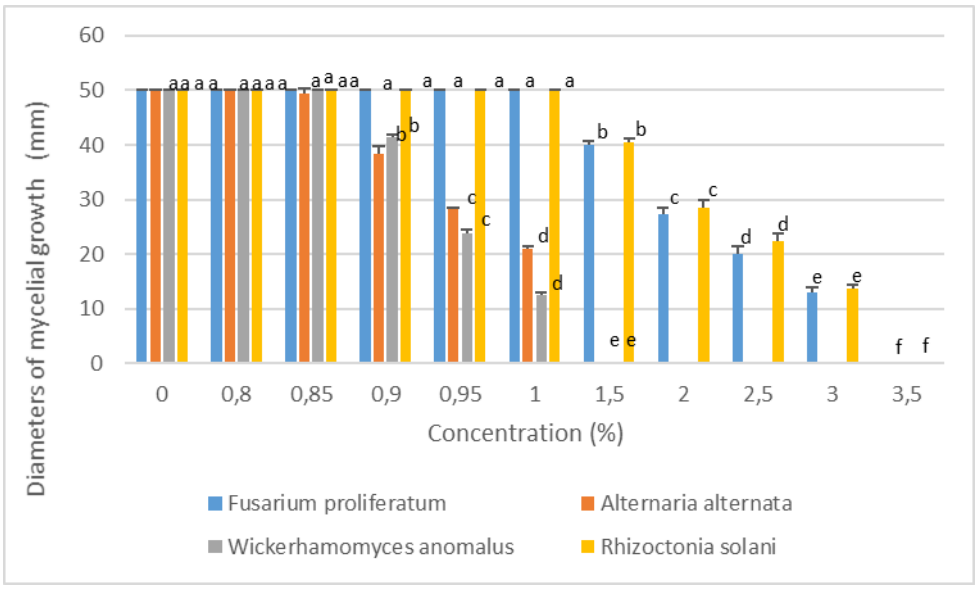

Figure 3: Effect of different concentrations of aqueous extracts of *Mentha pulegium* on mycelial growth.

According to **Figure 3**, we recorded a maximum diameter (50mm) of mycelial growth in the 4 untreated fungal strains (control) as well as at concentrations of 0.80% and 0.85%. The same

result was also obtained for *Fusarium proliferatum* and *Rhizoctonia solani* at concentrations of 0.90%, 0.95%, and 1%. The mycelial growth slightly decreases with increasing concentration of the aqueous extract of *Mentha pulegium*, eventually leading to complete inhibition of growth at 1.5% for *Alternaria alternata* and *Wickerhamomyces anomalus*, and at 3.5% for *Fusarium proliferatum* and *Rhizoctonia solani*. The Minimum Inhibitory Concentrations (MIC) .

3.5.4.*Ocimum basilicum*

The analysis of variance indicates a highly significant difference ($p<0.0001$) for the mycelial growth of the tested fungal strains.

Based on the results depicted in **Figure 4**, we observed a maximum mycelial growth diameter of 50mm in all fungal strains at concentrations of 0% (control) and 0.85%. This result is also observed for *Fusarium proliferatum* and *Rhizoctonia solani* in the range of [0-2.5%], as well as for *Rhizoctonia solani* at concentrations of 3% and 3.5%. As the concentration increases, the mycelial diameter of the fungal strains decreases, eventually leading to complete growth inhibition (0mm) at concentrations of 2% for *Wickerhamomyces anomalus*, 2.5% for *Alternaria alternata*, and 5.5% for *Fusarium proliferatum*. *Rhizoctonia solani* shows resistance up to a concentration of 7.5% (Minimum Inhibitory Concentration).

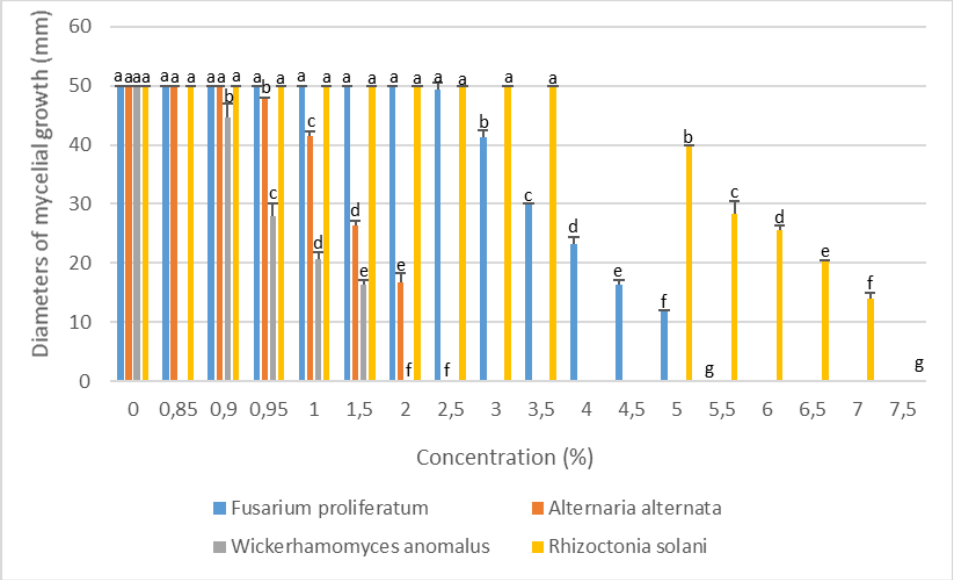

Figure 4: Effect of different concentrations of aqueous extracts of *Ocimum basilicum* on mycelial growth.

3.6. Antifungal Index

3.6.1. *Mentha spicata*

The action of the aqueous extract of *Mentha spicata* on the growth of different fungal strains is variable **Figure 5.** We observe an increase in the antifungal index as the concentration of the extract increases. Thus, we obtained average inhibition rates of 50.8%, 48.4%, 46.4%, and 41.6% at respective concentrations of 0.95%, 1%, 2.5%, and 3% for *Alternaria alternata*, *Wickerhamomyces anomalus*, *Fusarium proliferatum*, and *Rhizoctonia solani*, respectively. A

100% inhibition rate was observed at concentrations of 1.5%, 2%, 3.5%, and 4% for *Alternaria alternata*, *Wickerhamomyces anomalus*, *Fusarium proliferatum*, and *Rhizoctonia solani*, respectively.

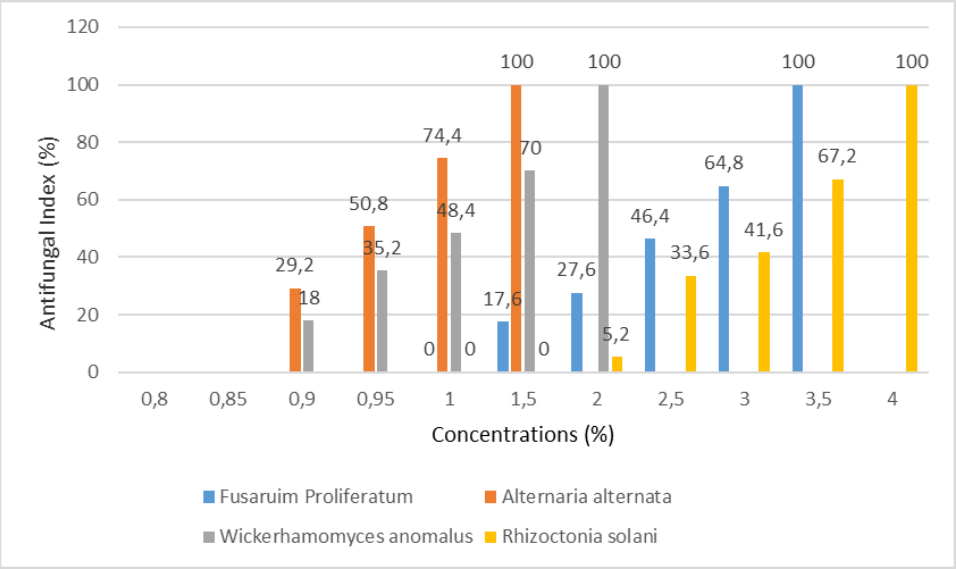

Figure 5: Antifungal Index showing inhibition of mycelial growth according to the concentration of aqueous extract of *Mentha spicata*.

3.6.2. *Mentha piperita*

Figure 6 represents the inhibition rate of mycelial growth for the tested fungal strains at different concentrations of aqueous extract of *Mentha piperita*. We observe that the antifungal index increases with the elevation of the extract concentration. An average inhibition rate of 64.8% and 60% is recorded at a concentration of 0.95% for *Alternaria alternata* and *Wickerhamomyces anomalus*, respectively. Regarding *Fusarium proliferatum* and *Rhizoctonia solani*, they exhibit respective average inhibition rates of 64.8% and 54% at a concentration of 3%. A total inhibition rate of 100% is recorded at concentrations of 1% for *Alternaria alternata* and *Wickerhamomyces anomalus*, and at 4% for *Fusarium proliferatum* and *Rhizoctonia solani*.

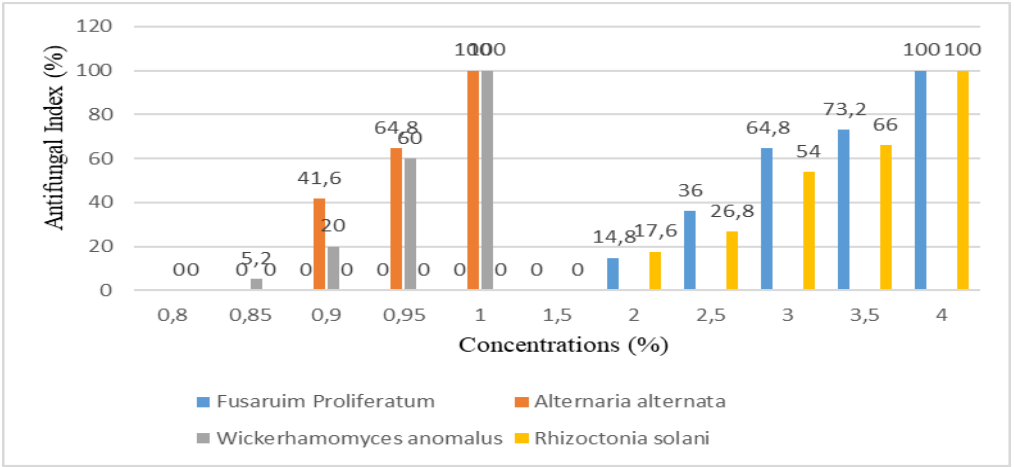

Figure 6: Antifungal Index showing inhibition of mycelial growth according to the concentration of aqueous extract of *Mentha piperita*.

3.6.3. *Mentha pulegium*

According to **Figure 7**, we observe an increase in the antifungal index as the concentration increases. We recorded respective average inhibition rates of 42.8% and 52.4% at a concentration of 0.95% for *Alternaria alternata* and *Wickerhamomyces anomalus*, and 59.8% and 55.2% at a concentration of 2.5% for *Fusarium proliferatum* and *Rhizoctonia solani*, respectively. A 100% inhibitory and fungicidal effect was observed at a concentration of 1.5% for *Alternaria alternata* and *Wickerhamomyces anomalus*. As for *Fusarium proliferatum* and *Rhizoctonia solani*, complete inhibition was only achieved at a concentration of 3.5% of the aqueous extract.

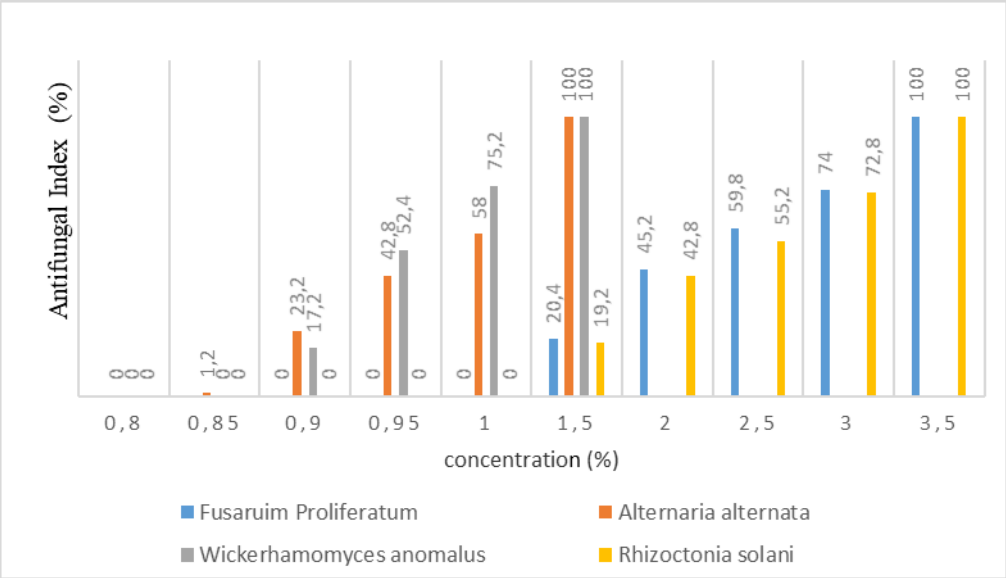

Figure 7: Antifungal Index showing inhibition of mycelial growth according to the concentration of aqueous extract of *Mentha pulegium*.

3.6.4. *Ocimum basilicum*

According to the results mentioned in **Figure 8**, we observe that the mycelial growth of fungi is inhibited with the increase in concentrations of the aqueous extract of *Ocimum basilicum.* Thus, we recorded average inhibition rates of 53.6%, 44%, 47.2%, and 49.2% at concentrations of 4%, 0.95%, 1.5%, and 6% respectively for *Fusarium proliferatum*, *Wickerhamomyces anomalus*, *Alternaria alternata*, and *Rhizoctonia solani*. As for the antifungal indices, we recorded inhibition rates of 100% at respective concentrations of 2%, 2.5%, 5.5%, and 7.5% for *Wickerhamomyces anomalus*, *Alternaria alternata*, *Fusarium proliferatum*, and *Rhizoctonia solani*.

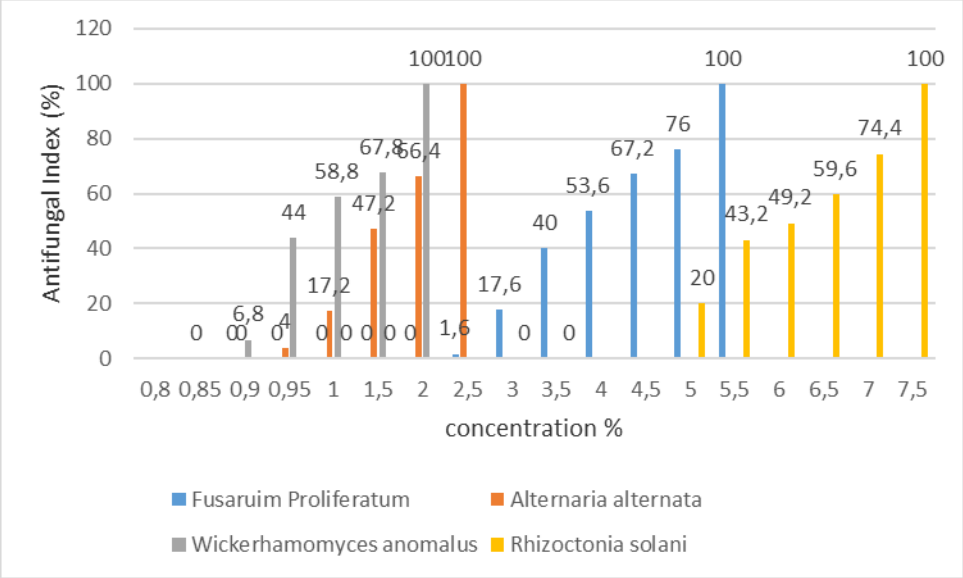

Figure 8: Antifungal Index showing inhibition of mycelial growth according to the concentration of aqueous extract of *Ocimum basilicum*.

The use of new natural substances as alternatives to chemical agents remains a promising solution for crop protection. The antifungal activities of the tested plant extracts against four fungal strains responsible for post-harvest diseases in potatoes are reported here for the first time.

The analysis of the effect of varying concentrations of the aqueous extracts of *Mentha spicata*, *Mentha piperita*, *Mentha pulegium*, and *Ocimum basilicum* on the mycelial growth of *Fusarium proliferatum*, *Alternaria alternata*, *Wickerhamomyces anomalus*, and *Rhizoctonia solani* strains showed that the activity of these plants is manifested at high concentrations. Depending on the plant species, a variation in activity ranging from 0% to 100% inhibition of mycelial growth can be observed. This demonstrates that the observed antifungal effects vary depending on the dose. Furthermore, this confirms that the bioactive compounds of the plants are perceived as substances that produce effective effects against pathogenic fungi **(Probavathy et al., 2006).**

With the increase in the concentration of the aqueous extract of *Mentha spicata*, a potent antifungal activity and an increase in the antifungal index of mycelial growth against the tested strains were obtained. According to a study conducted by **Ullah et al. (2011)** it was demonstrated that the aqueous extracts of *Mentha spicata* collected from four districts in Northern Khyber Pakhtunkhwa have inhibitory activity against *Trichophyton longifusus*, *Candida albicans*, and *Aspergillus flavus*. According to **Alaklabi et al. (2016),** the aqueous extract of *Mentha spicata* root exhibits a remarkable antifungal response against *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, and *Microsporum audouinii*.

In our study, the aqueous extract of peppermint (*Mentha piperita*) exhibits significant antifungal activity against the tested strains. Research conducted by **Ilboudo et al. in 2016**

confirmed that the extract of *M. piperita* demonstrated antifungal activity on the fourth day of incubation against *Phoma sorghina* and *Fusarium moniliforme*, and this activity increases with the concentration of the extract.

The aqueous extract of *M. pulegium* also had an effect on the tested strains. **El Khetabi et al. (2023)** demonstrated that the aqueous extract of this plant in Morocco possesses antifungal activity against the growth of *Monilinia laxa* and *Monilinia fructigena* with inhibition percentages of 90% and 58%, respectively. According to the results of **Alharbi et al. (2021)** the methanolic extracts of *M. rotundifolia* and *M. pulegium* exhibited moderate antifungal activity against the yeast *Candida albicans* and two species of fungi (*Aspergillus flavus* and *Aspergillus niger*).

The aqueous extract of basil (*Ocimum basilicum*) caused inhibition of mycelial proliferation in the studied fungal strains. **In 2019, Nugroho et al**. demonstrated the efficacy of the aqueous extract of sweet basil against *Sclerotium rolfsii* in Los Baños, Philippines, with an inhibition rate of 33.35%. According to the research conducted by **Jacob et al. in 2016**, the aqueous extracts of *O. basilicum* showed growth inhibition against *Fusarium oxysporum*.

Salem et al. (2021) mentioned that methanolic extracts of basil exhibit potential antifungal properties against **Candida albicans**, with an inhibition diameter ranging from 30 to 35 mm in both cultivation countries (Tunisia and Egypt).

It is likely that this activity is caused by the nature and molecular structure of the active components in the aqueous extracts. These compounds traverse the cell membrane, penetrate the cell interior, interact with key intracellular sites such as enzymes and proteins, and induce cell death **(Omidbeygi et al., 2007)**.

The extracts obtained from the aerial parts of the four plants used in this study were subjected to phytochemical screening and revealed the presence of various secondary metabolites, including tannins, saponins, and alkaloids.

The antimicrobial activity is associated with the chemical composition of phenolic substances, whose structure (aromatic ring associated with hydroxyl group in various positions) allows them to form hydrogen bonds with the SH groups in the active sites of target enzymes, resulting in the deactivation of these enzymes in fungi **(Ultee et al., 2002; Cheikna et al., 2011**). Within this group of compounds, **Harborne and Williams (2000)** as well as **Sepúlveda et al. (2012)** assert that the detected tannins and flavonoids are known for their ability to inhibit the growth of numerous microorganisms, including bacteria and fungi. Moreover, phenolic compounds, terpenes, and steroids, which have been identified, are selected as essential oils, defending the plant against fungi and bacteria **(Raven et al., 2000).** Likewise, phenolic terpenes also act by binding to the amine and hydroxylamine groups of microbial cell membrane proteins, causing alterations in permeability and leakage of intracellular contents **(Lopez-Malo et al., 2005)**. Thus, the presence of the identified compounds and their biological properties constitute the scientific basis for the significant antifungal activity of the plants following the antifungal tests.

4. Conclusion

The obtained results are promising and confirm the potential use of aqueous extracts of *Mentha spicata*, *Mentha piperita*, *Mentha pulegium*, and *Ocimum basilicum* as antifungal agents in biotechnology, opening up possibilities for applications in agriculture as biofungicides.

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