Trop J Nat Prod Res, August 2024; 8(8): 8019 - 8030 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antifungal Activity of Essential Oils and Aqueous Extracts of Three Moroccan Plants: A Study on Ten Phytopathogenic Fungi

Laila Reklaoui[1], Zine El Abidine Bzazou Elouazzani[1], Hassan Annaz[2], Manal Kasmi[1], Abderrazak Rfaki[3], Hassan Ghazal[3], Haiat Essalmani [1], Said Barrijal [1]*

[1]*Laboratory of Biotechnological Valorization of Microorganisms, Genomics, and Bioinformatics, Faculty of Sciences and Techniques, Abdelmalek Essaadi University, Tangier 90000., Morocco*
[2]*Agrobiosciences Program, College for Sustainable Agriculture and Environmental Science, Mohammed VI Polytechnic University, Ben-Guerir, 43150, Morocco*
[3]*National Center for Scientific and Technical Research CNRST, Rabat 10000, Morocco*

ARTICLE INFO

Article history:
Received 14 May 2024
Revised 30 May 2024
Accepted 09 August 2024
Published online 01 September 2024

ABSTRACT

In response to growing concerns regarding the environmental impact and the development of resistance to synthetic fungicides, researchers have undertaken investigations into natural alternatives for managing plant diseases caused by various fungi. This study investigates the antifungal properties of aqueous extracts and essential oils from Moroccan *Rosmarinus officinalis, Origanum compactum, and Origanum majorana* leaves against ten phytopathogenic fungi. The essential oils of the plants were extracted by hydrodistillation employing the Clevenger apparatus, and aqueous extracts were obtained through the infusion process. The antifungal efficacy of the extracts was assessed against ten phytopathogenic fungi utilising the poisoned food technique. The chemical composition of the essential oils was determined through GC-MS. Biochemical analyses were conducted to identify the phytochemical compounds responsible for the antifungal efficacy observed in the EOs and aqueous extracts. The essential oil extracts derived from *Origanum compactum and Origanum majorana* leaves exhibited potent antifungal properties against all tested fungi, with notably low IC_{50} values. While *Rosemary* essential oil displayed moderate activity. The aqueous extracts exhibited weak activities against all tested fungal strains. The GC-MS analysis revealed that 1.8-cineole accounted for 49.93% of the essential oil of *Rosemary*, pulegone (26.79%) of the essential oil of *Compact Oregano*, and carvacrol (61.66%) of the essential oil of *Marjoram.* The results of the biochemical analysis have shown that the extracted samples contain important components, specifically polyphenols, flavonoids, saponins, and catechic tannins. This study has showcased the promising potential of essential oils and aqueous extracts derived from Moroccan plants to effectively combat fungal diseases in plants.

Keywords: *Rosmarinus officinalis, Origanum compactum, Origanum majorana,* Essential oils, Antifungal activity, Gas Chromatography-Mass Spectrometry.

Introduction

Globally, there are an estimated 1.5 million species of fungi on earth, of which 10,000 are responsible for plant diseases. Unfortunately, some of these fungal organisms can cause considerable damage to plant crops and the post-harvest conservation of plant products. Fungal diseases account for 70% of all diseases affecting cultivated plants and result in billions of euros in losses each year on a global scale.[1] Many of these diseases have been known to cause famine, human health problems, ecosystem devastation, and even alter the course of human history.[2] Additionally, fungal pathogens have been found to produce mycotoxins such as ergotamine, deoxynivalenol (DON), and aflatoxin, further threatening human and livestock health.[3] Plant protection methods against these diseases rely heavily on phytosanitary products.[2]

*Corresponding author. Email: barrijal@yahoo.fr
Tel: +212 668 906 904

Citation: Reklaoui L, Elouazzani ZEB, Annaz H, Kasmi M, Rfaki A, Ghazal H, Essalmani H, Barrijal SP Antifungal Activity of Essential Oils and Aqueous Extracts of Three Moroccan Plants: a Study on Ten Phytopathogenic Fungi. Trop J Nat Prod Res. 2024; 8(8): 8019 – 8030 https://doi.org/10.26538/tjnpr/v8i8.14

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Chemical fungicides have undeniably contributed to the enhancement of agricultural yields. However, their excessive and improper application has given rise to long-term resistance issues and raised concerns about environmental impact and human health risks, particularly for farmers who use them in concentrated forms.

To address these challenges, significant efforts have been made to explore and develop alternative approaches, such as biofungicides, that are more ecologically friendly. These biofungicides involve the use of novel compounds extracted from plants that can restrict or suppress crop pests.[4] Notably, these biofungicides exhibit lower toxicity than chemical fungicides. Research has demonstrated that various compounds in plants and essential oils exhibit a wide range of activities, including antifungal, antibacterial, insecticidal, nematicidal, herbicidal, and antiviral properties.[5–12] Plants naturally produce secondary metabolites, such as flavonoids, saponins, alkaloids, tannins, and phenols, which are crucial to survival. These metabolites serve as a defence mechanism, enabling plants to protect themselves from herbivores, pathogens (such as bacteria, fungi, and viruses), and other plants. Additionally, these metabolites offer safeguards against adverse environmental conditions, such as harmful UV radiation, water loss, and low temperatures.[13] This breakthrough has facilitated a reduction in the excessive application of chemical fungicides in agriculture, leading to a mitigated adverse impact on the environment and human health.[14]

Morocco boasts an incredibly diverse plant fauna, making it a hub for the medicinal and aromatic plants (MAP) industry, which stands among the most abundant globally.

With a remarkable variety of 4,200 species, including 800 endemic species, Morocco possesses immense potential for developing and exporting nearly 400 species recognised for their medicinal and aromatic properties.[15] Within Morocco's rich repertoire of medicinal and aromatic plants, *Origanum compactum* stands out as an endemic species found in northern regions. Origanum compactum belongs to the Lamiaceae family, along with *Rosemary* and *Origanum majorana*; these plants are primarily known for their abundant phenolic compound content and are widely used in traditional Moroccan medicine to address various ailments, including diarrhoea, respiratory, skin, and urinary infections.[16] Plant extracts and essential oils present a highly promising alternative due to their extensive spectrum of action against a wide range of fungal species. Importantly, these alternatives do not pose risks to human health or cause environmental pollution.[17,18]

In this study, the antifungal activity of plant extracts was tested against ten destructive phytopathogenic fungi selected from diverse taxonomic groups. The fungi included *Botrytis cinerea, Aspergillus niger, Penicillium spp, Absidia glauca, Rhizoctonia solani, Rhizopus spp, Fusarium solani,* and three special forms of *Fusarium oxysporum*—*Fusarium oxysporum radicis lycopersici, Fusarium oxysporum lentis, and Fusarium oxysporum ciceria*. These fungi are commonly associated with postharvest diseases in tomatoes and other fruits, causing significant yield reductions ranging from 10% to 30% in major tomato crops.[19] The primary objective of recent research has been to foster the development of alternative control strategies to decrease reliance on synthetic fungicides. With this goal in mind, this study investigated the phytochemical properties and inhibitory potential of aqueous extracts and essential oils derived from *Origanum compactum, Origanum majorana,* and *Rosemary* leaves. The research specifically examined their effectiveness against ten phytopathogenic fungi.

Materials and Methods

Plant Collection and Identification

The samples of two wild plant species, *Rosmarinus officinalis*, and *Origanum majorana*, from two different regions of Morocco (Table 1) were collected in March 2021 and 2022 and then identified by Mr Bakkali Mohamed according to the catalog of vascular plants and the identification manuals for these plants.[20] *Origanum compactum* was sourced from the agricultural cooperative "Nouara Ain Lahjar" located in Ben Karrich, Tetouan region, North West of Morocco. This cooperative is accredited by the National Office for Food Security (ONSSA) under registration number 40.02.16, Lot number 9L1/21, where the plant is commercially cultivated. Fresh leaves of the plants were collected, cleaned, and air-dried in the dark at room temperature for three weeks to prevent photo-oxidation. Afterward, each dried plant was meticulously ground into a fine powder using a laboratory mixer.[21]

Table 1: List of plant species

Scientific Name	Vernacular name	Family	Part used	Place and year of collection	Latitude N	Longitude W
Rosmarinus officinalis	Azir	Lamiaceae	Leaf	Taza, March 2021 (Ribate El Kheir)	33°82'42.3	4°41'096
Origanum compactum	Za'atar	Lamiaceae	Leaf	Tetouan 2022 (Ben Karrich)	35°51'74.1	5°43'23.3
Origanum majorana	Mardadouch	Lamiaceae	Leaf	Tangier, March 2022	35°75'35	5°83'24.1

Extraction of Essential oils (EOs)

The powdered plant materials (200 g) were hydrodistilled using a Clevenger-type apparatus filled with 2 litres of distilled water for three hours. The resulting essential oils were carefully collected and stored in the dark at 4°C. The yield was quantified by calculating the amount of essential oil obtained per 100 grams of dry plant material.[22]

Aqueous extracts (AE)

To prepare the aqueous extracts, 16 grams of each plant's powdered material were first infused in 100 mL of distilled water at 100°C for 15 minutes. Following infusion, the mixture was allowed to macerate at room temperature for 24 hours. The macerated mixture was then filtered using Whatman No. 1 filter paper to remove larger plant debris. The filtrate was subsequently centrifuged at 4000 rpm for 10 minutes to further eliminate any remaining particulate matter. The supernatant was carefully collected and vacuum-filtered through a Millipore filter with 0.22 µm pore size to ensure the removal of fine particles. The resulting clear aqueous extract was stored in the dark at 4°C until further use.[23]

Source of plant pathogenic fungi

In this study, plant pathogenic fungi were obtained from stock cultures of the Laboratory of Biotechnological Valorisation of Microorganisms, Genomics, and Bioinformatics, Department of Biology, Faculty of Sciences and Techniques of Tangier, Abdelmalek Essaadi University, Morocco. The fungi were cultured on Petri dishes (90 mm) containing 20 mL of potato dextrose agar (PDA) and incubated at 23±2ºC for 7 days. After incubation, the viability of the fungal cultures was assessed by observing the growth and development of typical colony morphology and confirming spore production under a microscope. Several other plant pathogenic fungi were obtained from different host plants, as listed in Table 2. To ensure their purity and pathogenicity, the pathogen isolates were characterized based on the morphological characteristics of their mycelium, and their pathogenicity was tested by Koch's postulates. Prior to these tests, the viability of each isolate was confirmed by sub-culturing on fresh PDA plates and assessing their growth characteristics over 7 days at 23 ± 2ºC.

In-vitro antifungal activity of Essential oils

The antifungal activity of the essential oils was evaluated against phytopathogenic fungi using the food poisoning technique.[24] The oil was solubilised in tween 20 (0.1%), and ethanol (3%) was used as the surfactant. Before the experiment, the safety of the tween and ethanol mixture against pathogens was assessed. Different concentrations of essential oil were prepared from a stock solution and incorporated into a PDA medium maintained in supercooling at -45°C. After homogenisation, the mixture was poured into Petri dishes at 20 mL per dish. Circular implants of the tested plant pathogens, with a diameter of 5 mm, were inoculated aseptically onto the plates from 7-day-old fungal cultures. Control plates were prepared under the same conditions but without adding essential oil. The cultures were then incubated at 23°C ± 2 for 7 days for *Fusarium* spp. and 5 days for the other pathogens. Then, mycelial growth was estimated by measuring colony diameters in two perpendicular directions. The mean of these measurements was employed to determine the percentage inhibition of fungal growth relative to the control, using the following formula:

$$I(\%) = \frac{D - Di}{D}.100 \qquad \text{Equation 1}$$

Where:
I (%): percentage of mycelial growth inhibition
D: diameter of mycelial growth in a medium without the essential oil (control).
Di: diameter of mycelial growth in the presence of essential oil

Table 2: Sources of plant pathogenic fungi used for antifungal activity test

Plant pathogens	Class	Source	Disease
Fusarium oxysporum radicis lycopersici (Forl)	Ascomycetes	The mycotheque of our laboratory	Fusarium wilt of tomatoes
Fusarium oxyxporm lentis (Fol)	Ascomycetes	The mycotheque of our laboratory	Fusarium wilt of Lentils
Fusarium oxysporum ciceris (Foc)	Ascomycetes	The mycotheque of our laboratory	Fusarium wilt of chickpeas
Botrytis cinerea (BC)	Ascomycetes	The mycotheque of our laboratory	Gray mold
Aspergillus niger (AN)	Ascomycetes	The mycotheque of our laboratory	Black mold
Penicillium (Pen)	Ascomycetes	The mycotheque of our laboratory	Blue mold
Rhizopus (Rhizop)	Zygomycetes	The mycotheque of our laboratory	Rhizopus rot
Absidia glauca (AG)	Zygomycetes	The mycotheque of our laboratory	-
Fusarium solani (FS)	Ascomycetes	ENA	Root and fruit rot
Rhizoctonia solani (RS)	Basidiomycetes	ENA	Sheath rot

In-vitro antifungal activity of aqueous extracts
The aqueous extracts were added to the sterile liquid culture medium in suitable amounts to achieve final concentrations of 1, 2, 4, and 8 mg/mL. After the medium solidified, a 7-day-old mycelial explant of each pathogen was placed at the centre of each dish. The dishes were incubated at 23 ± 2°C, and mycelial growth was assessed after five or seven days by measuring the average diameter of the mycelial growth.

Determination of Minimum Inhibitory Concentration (MIC) and the concentration required to inhibit 50% of fungal growth (IC_{50})
The Minimum Inhibitory Concentration (MIC) was the lowest concentration at which no fungal growth was observed. The concentration required to inhibit 50% (IC_{50}) of fungal growth was calculated using regression equations.

Gas Chromatography-Mass Spectrometry (GC/MS) analysis
The analyses of the essential oil of the three plants were conducted using a Shimadzu Nexis 2030 gas chromatography (GC) instrument coupled with a TQ8040 NX mass spectrometer (MS) and equipped with a Restek RTX-5MS column (30 m length, 0.25 mm diameter, 0.25 µm film thickness). The initial temperature program commenced at 50°C for 2 minutes, followed by a gradual increase to 300°C at a rate of 5.5°C per minute, and was then held at 300°C for 3 minutes. Helium served as the carrier gas, flowing at a rate of 1.5 mL per minute. A split injection method was employed, where 1 µL of the sample was injected into the HTA 2800T injector at a temperature of 250°C. Mass spectrometric analysis was performed over the range of m/z 50-500. Identification of the essential oils relied on their retention indices (RIs), determined against a homologous series of C5-C24 (n-alkanes), and their mass spectra were compared with entries in the NIST and Wiley libraries, as reported in the literature.[25]

Phytochemical tests
The aqueous extracts were subjected to qualitative and quantitative analyses of specific secondary metabolites, identified through colourimetric and precipitation reactions.[26,27]

Quantitative analyses
Determination of Total Phenolic content
The total phenol content of the aqueous extracts was determined using the Folin-Ciocalteu assay.[26] Specifically, 200 µL of each extract was mixed with 1 mL of freshly prepared Folin-Ciocalteu reagent (diluted 10-fold) and 0.8 mL of 7.5% Na_2CO_3. After incubation at room temperature for 30 min, the absorbance of the resulting mixture was measured at 765 nm using a spectrophotometer.

Determination of flavonoid content
The aluminium trichloride colourimetric method was used to determine the aqueous extracts' flavonoid content.[27] 0.4 mL of distilled water was combined with 100 µL of the extract, and then 30 µL of a 5% sodium nitrite ($NaNO_2$) solution was added. 20 µL of a 10% $AlCl_3$ solution was added after five minutes. After letting the mixture rest for five minutes, 200 µL of a 1 M Na_2CO_3 solution and 250 µL of distilled water were added. The mixture was vortexed, and the absorbance was measured at 510 nm using a spectrophotometer. The results were expressed as milligram catechin equivalents per gram of dry plant material.

Statistical analysis
All experiments were conducted in duplicate, with three replicates each. The data were analysed using an analysis of variance (ANOVA) test, and differences between means were determined by the Tukey test at a significance level of 0.05. Statistical analysis was performed using Excel STAT version 2014 software.

Results and Discussion

GC-MS analyses of the EOs revealed 26 phytoconstituents in the EO of *Rosmarinus officinalis*, 39 components in *Origanum compactum* EO, and more than 40 components in *Origanum majorana* EO (Table 3 and Figure 1). *Rosmarinus officinalis* essential oil was found to be particularly rich in 1.8 cineole (49.93%), followed by camphor (13.16%) and *α*-pinene (6.28%). On the other hand, *majorana* EO was composed of carvacrol (61.66%), terpinen-4-ol (10.64%), and p-cymene (6.80%). *Origanum compactum* EO contained primarily pulegone and borneol (25.79% and 23.28%, respectively), as well as *α*-terpineol (9.86%) and carvacrol (5.79%). In this study, the analysis of the *Rosmarinus officinalis* essential oil (REO) chemical composition showed that 1.8-cineole, camphor, and *α*-pinene were the primary components, consistent with previous research findings[28–30] However,

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

the percentages of individual components in our REO (49.93%, 13.16%, and 6.28% for 1.8-cineole, camphor, and *α*-pinene, respectively) differed from those reported in other studies. For example, the percentages of these components in *rosemary* EOs varied widely (e.g., 43.99%-47.2% for 1.8-cineole, 12.41%-13.3% for camphor, and 10.09%-19.4% for *α*-pinene). Similarly, Elamrani *et al*.,[31] reported that 1.8-cineole (54.6%), camphor (8.6%), and *β*-pinene (6.8%) were the main components of *Rosmarinus eriocalix* oil.

Table 3: Chemical composition of the three essential oils determined by the GC-MS analysis

Compounds	**Retention Index**	**Relative abundance (%)**		
		Origanum compactum	*Origanum majorana*	*Rosmarinus officinalis*
***α*-Thujene**	918	0.22	0.29	-
***α*-Pinene**	925	3.20	0.20	6.28
Camphene	940	5.18	0.09	3.23
Sabinene	964	-	0.46	-
***β*-Pinene**	967	0.73	0.08	1.73
Octen-3-ol	969	-	-	0.32
Myrcene	984	0.21	0.55	0.87
3-octanol	984	0.41	-	-
***δ*-2-Carene**	996	-	-	0.63
***α*-Phellandrene**	997	0.08	0.08	0.41
***δ*-3-Carene**	1000	-	0.77	-
***α*-terpinene**	1006	0.40	-	-
p-Cymene	1014	2.99	6.80	2.65
Limonene	1020	0.96	-	2.41
Sylvestrene	1020	-	0.61	-
1,8-Cineole	1021	1.31	0.10	49.93
(Z)-*β*-Ocimene	1035	-	0.05	-
***γ*-Terpinene**	1048	0.97	2.19	0.40
cis-Sabinene hydrate	1060	-	0.53	-
ρ-Mentha-3,8-diene	1060	0.13	-	-
Terpinolene	1081	0.25	0.37	0.51
Linalool	1092	2.29	-	0.90
trans-Sabinene hydrate	1095	-	3.17	-
cis-ρ-Menth-2-en-1-ol	1117	-	0.53	-
***α*-Campholenal**	1123	-	0.06	-
trans-Pinocarveol	1136	0.14	-	-
trans-ρ-Menth-2-en-1-ol	1137		0.39	-
Camphor	1144	1.15	-	13.16
Pinocarvone	1164	-	-	0.27
Borneol	1167	23.28	1,10	5.08
cis-Pinocamphone	1177	-	-	0.28
Terpinen-4-ol	1179	-	10.64	1.53
ρ-Cymen-8-ol	1187	-	0.09	-
***α*-Terpineol**	1193	9.86	1.64	5.24
cis-Dihydro carvone	1196	-	0.12	-
Dihydro carveol	1197	0.09	-	-
cis-Piperitol	1198	-	0.16	-
Verbenone	1211	0.12	-	1.17
trans-Piperitol	1211	-	0.19	-

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

cis-ρ-Mentha-1(7),8-dien-2-ol	1223	-	0.04	-
Pulegone	1239	25.79	-	-
methyl ether Carvacrol	1247	1.61	0.09	-
Linalool acetate	1258	-	0.77	-
Carvenone	1263	0.16	0.07	-
trans-Ascaridol glycol	1273	-	0.17	-
Bornyl acetate	1291	1.59	-	-
Thymol	1296	2.19	0.47	-
Carvacrol	1305	5.79	61.66	0.27
Piperitenone	1346	1.41	-	-
Eugenol	1361	-	0.11	-
Carvacrol acetate	1374	-	0.08	-
***α*-Ylangene**	1378	-	-	0.28
***α*-Copaene**	1379	0.23	-	-
Geranyl acetate	1383	-	0.09	-
***β*-Bourbonene**	1389	0.15	0.05	-
(E)-caryophyllene	1422	4.57	0.91	1.08
***α*-trans-Bergamotene**	1434	-	0.12	-
Aromadendrene	1439	-	0.16	-
***α*-Humulene**	1453	0.28	-	0.29
allo-Aromadendrene	1459	0.14	-	-
***γ*-Muurolene**	1472	0.12	0.07	-
***β*-Bisabolene**	1499	-	1.82	-
***γ*-Cadinene**	1508	0.37	0.22	0.50
***δ*-Cadinene**	1518	0.46	0.16	-
Spathulenol	1582	-	0.41	-
Caryophyllene oxide	1589	0.76	0.70	0.57
epi-*α*-Cadinol	1643	0.16	0.48	-

In commercially available EO from *R. officinalis*, the main constituents are 1.8-cineole (19.59%), camphor (18.35%), *α*-pinene (17.17%), camphene (10.10%), *β*-pinene (6.08%), and limonene (3.90%).[32] These proportions differ from our findings, suggesting that the chemical composition of REO may vary depending on the geographic location and other factors. The chemical composition of *Origanum Compactum* differed slightly from those reported in the literature, with carvacrol and thymol dominating as a significant component.[33] The chemical composition of *Origanum majorana* EO in this study was close to that reported by several studies conducted in Morocco.[34]

The EO was characterised by its oxygenated monoterpenes (terpinene-4-ol and trans-sabinene hydrate) and non-oxygenated monoterpenes (mainly *γ*-terpinene and *α*-terpinene) and carvacrol. According to the literature, the yield and chemical composition of EO change depending on the harvesting period, extraction method, and the drying of the plant, therefore our results are consistent with previous work of Aimad *et al*.,[35] In the antifungal screening, the essential oils inhibited all the fungi in a dose-dependent manner, as shown in Table 4 and Figure 2. The three essential oils demonstrated partial or complete inhibitory activity against all the tested fungi. Moreover, the *Origanum compactum* and *majorana* were the most effective, showing high antifungal activity

with more than 85% inhibition of all the fungi at 0.25% to 0.5%. Still, only the *Origanum compactum* EO significantly inhibited mycelial growth in all the fungi at low doses (0.05%). In general, the degree of antifungal activity was attributed to the chemical composition of the essential oil. However, this activity may also depend on the compounds that act synergistically or antagonistically. The results of the present study indicate that the essential oils of *Rosmarinus officinalis*, *Origanum compactum*, *and majorana* exhibit significant antifungal activity against all the tested fungi. *Rosmarinus officinalis* EO exhibited less antifungal activity at a concentration of 0.25% with an estimated IC_{50} of 0.15 - 0.44%. In comparison, both *Origanum* EO showed high antifungal activity for the same concentration (more than 50% for all the fungi) and IC_{50} values of 0.027% and 0.24%, respectively. The study revealed that *Rhizoctonia solani* was overly sensitive to all the EOs tested, with the most effective inhibition observed with *Origanum compactum and majorana* (IC_{50} level of <0.05%). In contrast, *Fusarium solani* showed lower sensitivity to EOs, with high IC_{50} = 0.44% observed with *Rosmarinus officinalis*. *Origanum compactum* was fungicidal at 0.05%, followed by *Origanum majorana* and *Rosemary* at 0.25% and 0.5%, respectively. recorded for *Origanum compactum* EO for all the fungi, followed by *Origanum majorana* and r*osemary* (Table 5).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: Antifungal activity of essential oils in percentage inhibition percentage

		Percentage of radial growth inhibition Plant and strain pathogens									
EO	**%**	**Forl**	**Foc**	**Fol**	**FS**	**BC**	**Rhizop**	**AN**	**RS**	**Pen**	**AG**
R. off	0	0	0	0	0	0	0	0	0	0	0
	0.05	24.35±0.00^{c}	36.31±1.03^{c}	39.07±1.15^{b}	7.14±1.19^{a}	25.31±1.24^{a}	0.00±0.00nd	26.67±1.92^{b}	20.33±1.41^{b}	31.11±1.92^{a}	11.11±0.00^{b}
	0.1	39.13±8.49^{c}	46.43±0.00^{a}	51.66±1.15^{bc}	10.32±0.69^{a}	26.14±0.72^{a}	0.00±0.00nd	37.41±1.28^{b}	27.64±1.41^{b}	35.19±3.21^{a}	20.37±1.70^{b}
	0.25	72.61±2.26^{a}	47.02±1.03^{a}	64.24±1.99^{bc}	24.21±1.82^{ab}	40.24±0.00^{b}	94.44±0.00nd	45.93±1.28^{b}	87.80±0.00^{c}	51.85±3.21^{b}	77.41±0.64^{c}
	0.3	73.04±6.16^{a}	91.07±0.00^{d}	90.07±0.00^{c}	30.95±2.38^{b}	75.93±0.72^{bc}	94.44±0.00nd	51.11±1.92^{b}	87.80±0.00^{c}	94.44±0.00^{d}	92.96±0.64^{a}
	0.5	83.48±0.76^{a}	91.07±0.00^{d}	90.07±0.00^{c}	57.54±1.82^{b}	82.57±1.24^{c}	94.44±0.00nd	94.44±0.00^{d}	87.80±0.00^{c}	94.44±0.00^{d}	94.44±000^{a}
O. com	0	0	0	0	0	0	0	0	0	0	0
	0.05	49.05±0.00nd	32.14±0.88^{b}	90.07±0.00nd	48.15±2.42^{b}	80.08±1.25^{c}	94.44±0.00nd	27.78±0.00^{b}	87.80±0.00nd	30.37±2.79^{b}	38.15±4.21^{b}
	0.1	75.19±0.00nd	92.35±0.00^{d}	90.07±0.00nd	73.02±1.59^{bc}	93.78±0.00^{d}	94.44±0.00nd	39.26±2.79^{b}	87.80±0.00nd	35.93±2.80^{bc}	94.44±0.00^{d}
	0.25	88.72±0.00nd	92.35±0.00^{d}	90.07±0.00nd	88.89±0.00^{c}	93.78 ±0.00^{d}	94.44±0.00nd	50.74±0.64^{bc}	87.80±0.00nd	94.44±0.00^{d}	94.44±0.00^{d}
	0.3	88.72±0.00nd	92.35±0.00^{d}	90.07±0.00nd	92.06±0.00^{d}	93.78±0.00^{d}	94.44±0.00nd	54.82±1.29^{bc}	87.80±0.00nd	94.44±0.00^{d}	94.44±0.00^{d}
	0.5	88.72±0.00nd	92.35±0.00^{d}	90.07±0.00nd	92.06±0.00^{d}	93.78±0.00^{d}	94.44±0.00nd	94.44±0.00^{d}	87.80±0.00nd	94.44±0.00^{d}	94.44±0.00^{d}
O. maj	0	0	0	0	0	0	0	0	0	0	0
	0.05	47.13±1.99^{bc}	92.35±0.00nd	79.74±18.12^{a}	92.89±0.00nd	86.72±1.90^{a}	0.00± 0.00nd	30.74±2.79^{b}	87.80±0.00nd	32.22±1.92^{b}	38.15±4.21^{b}
	0.1	70.88±1.75^{c}	92.35±0.00nd	90.20±0.00^{a}	92.89±0.00nd	87.97±1.90^{a}	0.00±0.00nd	41.11±1.92^{bc}	87.80±0.00nd	37.04±3.21^{b}	94.44±0.00^{d}
	0.25	94.25±0.00^{d}	92.35±0.00nd	90.20±0.00^{a}	92.89±0.00nd	93.78±0.00^{d}	94.44±0.00nd	51.48±1.70^{a}	87.80±0.00nd	94.44±0.00^{d}	94.44±0.00^{d}
	0.3	94.25±0.00^{d}	92.35±0.00nd	90.20±0.00^{a}	92.89±0.00nd	93.78±0.00^{d}	94.44±0.00nd	55.56±0.00^{a}	87.80±0.00nd	94.44±0.00^{d}	94.44±0.00^{d}
	0.5	94.25±0.00^{d}	92.35±0.00nd	90.20±0.00^{a}	92.89±0.00nd	93.78±0.00^{d}	94.44±0.00nd	94.44±0.00^{d}	87.80±0.00nd	94.44±0.00^{d}	94.44±0.00^{d}

*Numbers followed by different letters denote statistical significance according to Fisher's LSD test (p< 005)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

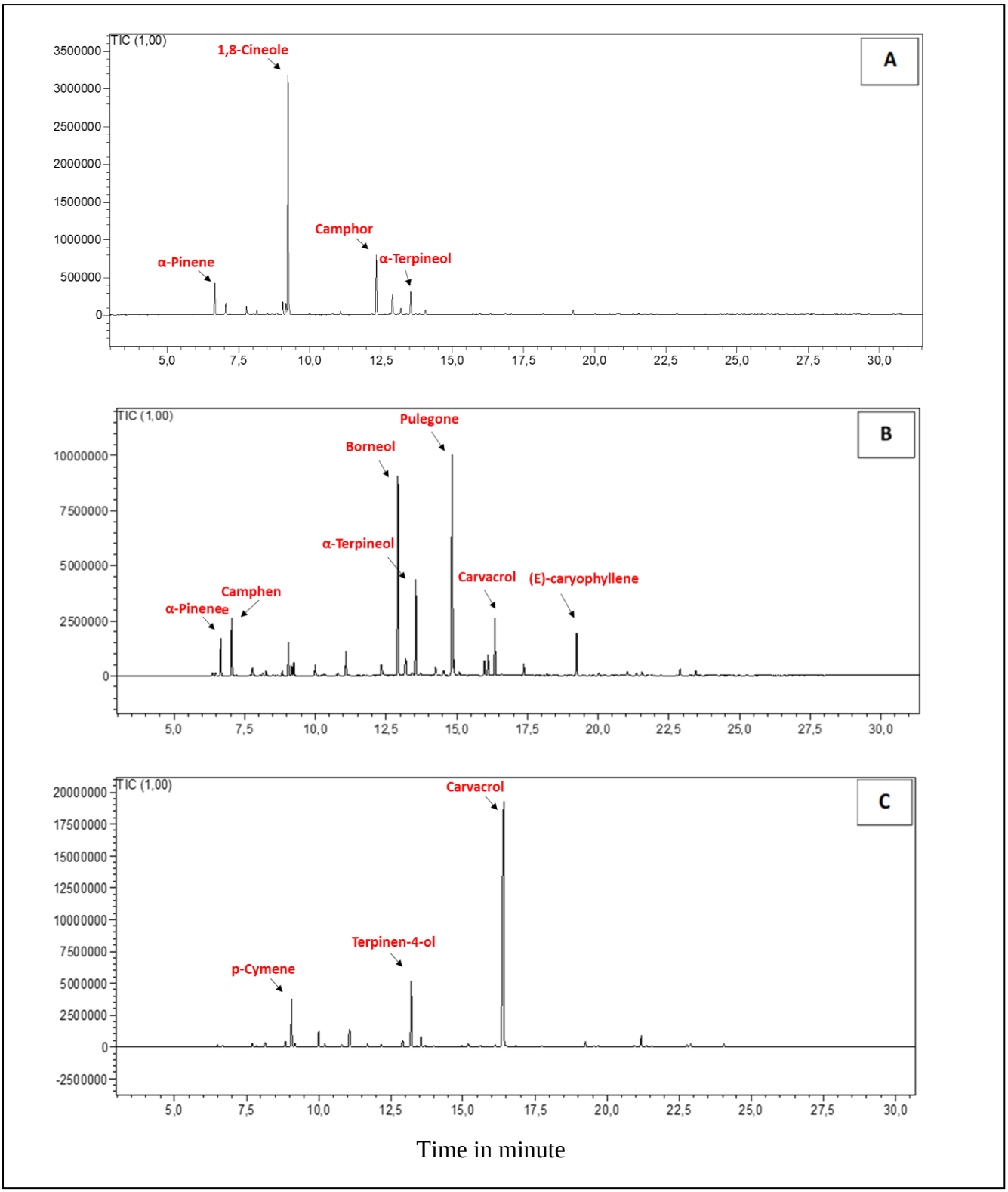

Figure 1: GC/MS Chromatograms of essential oil of **A**: *Rosmarinus officinalis* **B**: *Origanum compactum* **C**: *Origanum majorana*

Furthermore, using probit analysis, the effective concentration (IC_{50}) values for each fungus were determined. The lowest IC_{50} values were It was noted that the efficacy of these three essential oils from the *Lamiaceae* family exhibited variability across the ten fungi assessed, contingent upon their concentration and composition. Furthermore, the sensitivity of fungal isolates to different EOs varied, which could be attributed to the fungal cell wall composition and the efficacy of the EOs. It is imperative to acknowledge that the methodology employed in testing the essential oils may influence the results owing to their volatile and hydrophobic nature. The lipophilic nature of EOs may facilitate their penetration of fungal membranes, leading to membrane disruption.[36,37] Therefore, it is important to consider the method used for assessing the efficacy of EOs. Previous studies have shown that the volatile fraction of essential oils exhibits superior antifungal activity against the phytopathogens tested compared to the direct contact method.[38] This suggests that the volatile compounds of EOs may play a

crucial role in their antifungal activity. Numerous studies have demonstrated that the fungicidal activity of plant essential oils can be attributed to their active components, such as phenols, aldehydes, and ketones.[39]

This study found lower MIC values for *O. compactum* compared to previous studies against *A. niger, A. alternata, B. cinerea, P. digitatum, P. italicum, V. dahlea, and P. expansum*.[40] According to a recent study, *Origanum majorana's* minimum inhibitory concentration (MIC) against pathogenic fungi, such as Candida species, varied from 58 to 468 g/mL.[41] Moreover, Bouyahya *et al*., [42] discovered a rough correlation between elevated levels of thymol, carvacrol, α- and γ-terpinene, and p-cymene and their biological activity both *in vitro* and *in vivo*. Earlier studies have looked at the mechanism of action of EOs in fungi for a better understanding. The antifungal effect of *Origanum* might be partly attributed to EO terpenes and phenolic compounds, which are involved in cell membrane damage, cellular material leakage, inhibition of electron transport, and ATPase in the mitochondria. All of these processes result in the death of microorganisms.[43]

Table 5: Minimum inhibitory concentration (MIC) and IC_{50} inhibitory concentration values against radial growth of ten fungi

	Essential oils					
Plant pathogens	*Rosmarinus officinalis*		*Origanum compactum*		*Origanum majorana*	
	IC_{50} (%)	MIC (%)	IC_{50} (%)	MIC (%)	IC_{50} (%)	MIC (%)
Fusarium oxysporum radicis lycopersici	0.15	0.50	0.05	0.25	0.06	0.25
Fusarium oxyxporm lentis	0.09	0.3	0.03	0.05	0.03	0.1
Fusarium oxysporum ciceris	0.25	0.3	0.06	0.1	0.03	0.05
Botrytis cinerea	0.26	0.5	<0.05	0.1	<0.05	0.25
Aspergillus niger	0.29	0.5	0.24	0.5	0.23	0.5
Penicillium	0.23	0.3	0.14	0.25	0.13	0.25
Rhizopus	0.18	0.25	<0.05	0.05	0.18	0.25
Absidia glauca	0.18	0.3	0.06	0.1	0.06	0.1
Fusarium solani	0.44	--	0.05	0.3	<0.05	0.05
Rhizoctonia solani	0.16	0.25	<0.05	0.05	<0.05	0.05

In vitro antifungal screening results of the three aqueous extracts studied (*Rosmarinus officinalis, Origanum compactum,* and *majorana*) showed that the extracts exerted a dose-dependent antifungal effect on the mycelial growth of the ten fungi, but with varied concentrations. Except for *Rhizopus*, the aqueous extracts showed lower antifungal activity than the essential oils. The three aqueous extracts exhibited less than 50% inhibition on all the fungi at a higher concentration (8 mg/mL). However, *Rosmarinus officinalis* aqueous extract was the most active against *Fusarium oxysporum* species (Table 6) compared with *Origanum* extracts, which showed a low inhibitory capacity against mycelial growth of the ten fungi. It would be interesting to investigate the active compounds in the *Rosmarinus officinalis* aqueous extract that contribute to its antifungal activity against *Fusarium oxysporum.*

These findings are consistent with a recent study by Elkhatabi *et al*.,[44] that showed that a lower concentration of pomegranate peel aqueous extract inhibited the spore germination of *Monilinia spp*. more effectively than mycelial growth. This is also in agreement with the study by Aourach *et al*.,[45] which demonstrated that aqueous extracts exhibited high inhibitory levels against conidial germination and sporulation compared to mycelial growth, indicating that the inhibitory components in the extracts could limit pathogen dissemination. Furthermore, the study by Kasmi *et al*.,[46] confirmed these results, demonstrating that aqueous extracts significantly affect sporulation intensity, which plays a crucial role in disseminating the fungus. Specifically, the extracts of *Thymus vulgaris, Cymbopogon citratus,* and *Lavandula officinalis* showed an inhibition percentage of *Botrytis cinerea* sporulation higher than 60% at a concentration of 8 mg/mL. In the phytochemical screening of the studied plants, the results revealed that the three MAPs have different classes of secondary metabolites that are either lipophilic or hydrophilic, but the use of water as a polar solvent allows the extraction of phenolic compounds, mainly flavonoids.

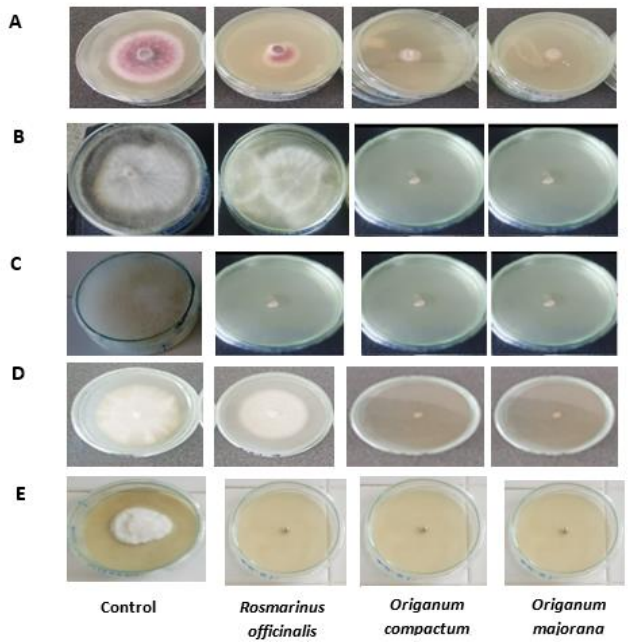

Figure 2: The activity of *Rosmarinus officinalis, Origanum compactum,* and *majorana* essential oils (0.25%) on the mycelial growth of *Fusarium oxysporum radicis lycopersici* **(A)** *Botrytis cinerea* **(B)** *Rhizopus* **(C)** *Fusarium solani* **(D)** and *Rhizoctonia solani* **(E).**

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 6: Antifungal activity of aqueous extracts inhibition percentage

		Percentage of radial growth inhibition									
		Plant and strain pathogens									
AE	**mg/ mL**	**Forl**	**Foc**	**Fol**	**FS**	**BC**	**Rhizop**	**AN**	**RS**	**Pen**	**AG**
R. off	0	0	0	0	0	0	0	0	0	0	0
	1	38.10±2.07^{a}	47.06±0.00^{a}	35.71±0.00nd	18.93±0.84^{a}	2.59±0.64^{a}	0.00±0.00nd	0.00±0.00^{a}	16.89±2.03^{a}	6.25±0.00^{c}	8.96±1.71^{a}
	2	39.29±3.58^{a}	47.06±0.00^{a}	35.71±0.00nd	18.93±0.84^{a}	3.70±0.64^{a}	0.00±0.00nd	2.35±2.36^{a}	18.92±2.03^{a}	12.50±0.00^{a}	10.45±1.12^{a}
	4	40.48±2.06^{a}	49.02±1.70^{ab}	39.29±0.00nd	19.42±0.84^{a}	3.70±0.64^{a}	0.00±0.00nd	5.10±0.68^{a}	20.27±1.17^{a}	15.00±0.00^{a}	11.57±1.12^{a}
	8	41.67±2.06^{a}	50.00±0.00^{ab}	42.86±0.00nd	23.79±3.03^{b}	4.07±0.64^{a}	0.00±0.00nd	6.67±1.36^{a}	21.62±1.17^{a}	17.50±2.17^{a}	11.94±1.71^{a}
O. com	0	0	0	0	0	0	0	0	0	0	0
	1	10.66±0.88^{b}	11.88±0.86^{a}	23.91±2.18a	0.50±0.87^{a}	0.00±0.00^{b}	0.00±0.00nd	0.79±0.68^{a}	15.75±2.06^{a}	26.67±1.44^{a}	9.26±2.31^{a}
	2	15.73±0.88^{b}	12.38±2.57^{a}	25.36±1.26a	1.50±0.87^{a}	0.00±0.00^{b}	0.00±0.00nd	3.92±1.36^{a}	19.18±1.18^{a}	28.33±3.15^{a}	11.48±0.64^{a}
	4	26.39±0.88^{bc}	12.87±2.27^{a}	26.81±1.25a	2.00±0.87^{a}	2.59±0.64^{a}	0.00±0.00nd	5.10±0.68^{a}	21.92±2.06^{a}	35.83±4.02^{a}	13.70±2.31^{a}
	8	31.47±1.52^{c}	14.85±3.74^{a}	28.98±1.25a	3.50±0.87^{a}	4.07±1.70^{a}	0.00±0.00nd	7.84±1.80^{a}	22.60±1.18^{a}	42.50±2.17^{a}	2296±3.57^{b}
O. maj	0	0	0	0	0	0	0	0	0	0	0
	1	14.51±1.56^{b}	11.88±0.86^{a}	16.18±2.21^{a}	0.50±0.87^{a}	0.00±0.00^{b}	0.00±0.00nd	0.39±0.68^{a}	15.75±2.06^{a}	25.83±1.44^{b}	9.26±2.31^{a}
	2	22.28±1.55^{b}	12.38±2.57^{a}	19.85±2.55^{a}	1.50±0.87^{a}	0.00±0.00^{b}	0.00±0.00nd	3.14±1.36^{a}	19.18±1.18^{a}	31.25±0.00^{c}	11.48±0.64^{a}
	4	30.57±0.90^{a}	12.87±2.27^{a}	25.0±2.21^{a}	2.00±0.87^{a}	3.70±0.64^{a}	0.00±0.00nd	5.49±0.68^{a}	21.92±2.06^{a}	38.33±0.72^{a}	13.70±2.31^{a}
	8	32.13±0.89^{a}	14.85±3.74^{a}	27.94±1.27^{a}	3.50±0.87^{a}	4.81±0.65^{a}	0.00±0.00nd	7.45±1.36^{a}	22.60±1.18^{a}	41.25±2.17^{a}	22.96±3.57^{b}

*Numbers followed by different letters denote statistical significance according to Fisher's LSD test ($p < 005$)

According to the results in Table 7, all the aqueous extracts studied contain Gallic tannins with a higher concentration. Notably, saponins were more concentrated in the aqueous extracts of *Rosmarinus officinalis*. Saponins are known to interact with sterols, proteins, and phospholipids in fungal cell membranes, causing a loss of structural integrity of the cell membrane and an increase in ionic permeability,[46] which confirms the inhibitory efficacy of the aqueous extract of *Rosmarinus officinalis*.

The results in Table 8 indicate a significant difference in the total phenols content in the aqueous extract of *Origanum compactum* (0.9 mg/mL) compared to the other aqueous extracts. Flavonoids represent a small proportion, not exceeding 12% of total phenols for all extracts, except for *Origanum compactum*, which contains a higher flavonoid content than the other two extracts. The flavonoid class is the primary active constituent of the aqueous extracts and may directly contribute to their antimicrobial activities. The use of synthetic chemicals for disease control is becoming increasingly restricted due to their potential carcinogenicity, high residual toxicity, long degradation period, and environmental pollution. Furthermore, the emergence of fungal populations with various fungicide resistance resulting from repeated use of chemical fungicides has complicated disease management.[47] Therefore, plant extracts such as essential oils and aqueous extracts are considered alternative biological control methods due to their inhibitory activity on pathogen growth, either as main components or as antimicrobial adjuvants.[48]

Table 7: Tannin and saponin contents of the aqueous extracts

Aqueous extracts	Tannins		Saponins
	Gallic tannins	Catechic tannins	
Rosmarinus officinalis	+	-	+
Origanum compactum	+	-	+
Origanum majorana	+	-	+

–: Negative test; +: Positive test.

Table 8: Total polyphenol and flavonoid contents of the aqueous extracts

Aqueous extracts	Total phenol content (Mg gallic acid equivalents/mL) (C)	flavonoid content (Mg of catechin equivalents/mL) (R)	Flavonoid-to-phenol ratio ((R)/(C)) x100
Rosmarinus officinalis	0.56 ± 0.08	0.06 ± 0.01	10.71%
Origanum compactum	0.9 ± 0.06	0.1 ± 0.02	11.11%
Origanum majorana	0.69 ± 0.06	0.07 ± 0.01	10.14%

Flavonoid content (R); Total phenol content (C); Flavonoid-to-phenol ratio (R) / (C)

Conclusion

Several investigations have explored plant substances as natural alternatives to synthetic fungicides against fungal pathogens. This study assesses three plant extracts for antifungal activity against ten phytopathogenic fungi. The essential oils of *Origanum compactum* and *majorana* were the most effective, followed by *Rosmarinus officinalis*. Essential oils inhibited mycelial growth more than aqueous extracts, which may be stronger against fungal sporulation. These extracts contained antifungal compounds, such as saponins, terpenes, flavonoids, alkaloids, phenols, and steroids, proving safe and viable alternatives to traditional fungicides.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We thank Abdelaaziz Farhaoui of the National School of Agriculture for providing *Rhizoctonia solani* and *Fusarium solani* fungi strains.

References

1. Fisher AC, Hanemann WM, Roberts MJ, Schlenker W. The Economic Impacts of Climate Change: Evidence from Agricultural Output and Random Fluctuations in Weather: Comment. Am Econ Rev. 2012 Dec 1;102(7):3749–60.
2. Agrios GN. Plant pathology. 5th ed., [3rd print]. Amsterdam: Elsevier Academic Press; 2008. 922 p.
3. Wu F. Perspective: Time to face the fungal threat. Nature. 2014 Dec;516(7529):S7–S7.
4. Thakore H, McMahon T. An interactive e-tutorial in pathology. Med Educ. 2006 Nov;40(11):1135.
5. Chang SJ, Chang YC, Lu KZ, Tsou YY, Lin CW. Antiviral Activity of *Isatis indigotica* Extract and Its Derived Indirubin against Japanese Encephalitis Virus. Evid Based Complement Alternat Med. 2012;2012:1–7.
6. Dulger B, Hacioglu N. Antifungal Activity of Endemic *Salvia tigrina* in Turkey. Trop J Pharm Res. 2008 Sep 11;7(3):1051–4.
7. Gökçe A, Whalon ME, Çam H, Yanar Y, Demirtaş İ, Gören N. Plant extract contact toxicities to various developmental stages of Colorado potato beetles (Coleoptera: Chrysomelidae). Ann Appl Biol. 2006 Oct 1;149(2):197–202.
8. Hosein E, Sabokbar A, Kordbacheh P, Bahonar A, Bayat M, Saeednejad L. Antifungal effect of *Zataria multiflora*: An in vitro evaluation. Glob Vet. 2010 Jan 1;4:140–3.
9. Kepenekci İ. Effects of Some Indigenous Plant Extracts on Mortality of the Root Lesion Nematode, Pratylenchus thornei Sher & Allen. Egypt J Pest Control. 2016 Jan 1;26:119–21.
10. Kordali S, Cakir A, Akcin TA, Mete E, Akcin A, Aydin T, Kilic H. Antifungal and herbicidal properties of essential oils and n-hexane extracts of *Achillea gypsicola* Hub-Mor. and *Achillea biebersteinii Afan*. (Asteraceae). Ind Crops Prod. 2009 Mar;29(2–3):562–70.
11. Kordali S, Usanmaz Bozhüyük A, Cakir A, Cavusoğlu A, Ercisli S. In Vitro Antifungal Effect of Essential Oils from *Nepeta meyeri* Benth. Egypt J Biol Pest Control. 2013 Jan 1;23:209–13.

12. Onaran A. Antifungal activity of *Trachystemon orientalis L*. aqueous extracts against plant pathogens. J Food Agric Environ Vol10 34 287-291 2012. 2012;
13. Kong CH, Wang P, Xu X. Allelopathic interference of *Ambrosia trifida* with wheat (*Triticum aestivum*). Agric Ecosyst Environ. 2007 Mar 1;119:416–20.
14. Corrêa-Junior D, Parente CET, Frases S. Hazards Associated with the Combined Application of Fungicides and Poultry Litter in Agricultural Areas. J Xenobiotics. 2024 Mar;14(1):110–34.
15. Naggar M, Iharchine K. For a Sustainable Valorization of Non-Timber Forest Products: Case of *Rosemary* Facies in the Oriental Region (Morocco). In Durban, Afrique du Sud; 2015. p. 8.
16. Bellakhdar J. The Traditional Moroccan Pharmacopeia – Ancient Arab Medicine and Popular Knowledge. 2nd ed. Paris: Ibis Press; 1997.
17. Wang Y, Zhang X, Zhou JY, Li XC, Min J, Lei TF, Zhou T. Inhibitory Effects of Five Antifungal Substances on Development of Postharvest Pathogen *Rhizopus oryzae*. J Agric Biotechnol. 2015 Jan 4;23(1):107–17.
18. Zhang Z, Yang T, Mi N, Yong W, Li G, Wang L, Xie Y. Antifungal activity of monoterpenes against wood white-rot fungi. Int Biodeterior Biodegrad. 2016 Jan 31;106:157–60.
19. Etebu E, Nwauzoma AB, Bawo DDS. Postharvest Spoilage of Tomato (*Lycopersicon esculentum Mill*.) and Control Strategies in Nigeria. J Biol. 2013;
20. Fennane M (1957) A. Practical Flora of Morocco: Manual for the Identification of Vascular Plants. Scientific Institute, Mohammed V University - Agdal; 1999.
21. Kasmi M, Essalmani H. Antifungal Effect of *Rosmarinus officinalis L*. and *Salvia officinalis L*. Extracts against Tomato Grey Mould. Trop J Nat Prod Res TJNPR. 2022 Feb 1;6(2):180–7.
22. Jilali SBE, Rachid I, Ghada B, Tarik M, Sanae R, Abderrazzak K. Effect of Isolation Techniques on the Quantity, Quality, and Antimicrobial Activity of *Lavandula dentata* Essential Oils. Trop J Nat Prod Res TJNPR. 2023 May 1;7(4):2713–7.
23. Ogba OM, Udoh DI, Udonkang MI, Eyo AAO, Chukwueke SE, Eshemitan Z, Akpan NG. Antimicrobial Effect of *Cassia alata* Leaf Extracts on Fungal Isolates from Tinea Infections. Trop J Nat Prod Res TJNPR. 2023 Jul 2;7(6):3034–7.
24. Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK. Assessment of *Thymus vulgaris L*. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. Innov Food Sci Emerg Technol. 2008 Oct 1;9(4):575–80.
25. Adams RP. Identification of Essential Oil Components By Gas Chromatography-Mass Spectrometry. 4th edition. Carol Stream, Ill: Allured Pub Corp; 2017. 804 p.
26. Yumita A, Hikmawanti NPE, Hanani E, Saputri CW, Hanana PH, Ero JND, Marcelena M, Baytisani T, Sofiana FA, Shania AF, Saputri ESA, Islami FPN. Exploring the Polyphenol Contents and Antioxidant Capacity of the Leaf Extracts of Selected Indonesian *Syzygium* Species. Trop J Nat Prod Res TJNPR. 2023 Jul 2;7(6):3119–24.
27. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY. Quantification of Polyphenolics and Their Antioxidant Capacity in Fresh Plums. J Agric Food Chem. 2003 Oct 1;51(22):6509–15.
28. Ait-Ouazzou A, Lorán S, Bakkali M, Laglaoui A, Rota C, Herrera A, Pagán R., Conchello P. Chemical composition and antimicrobial activity of essential oils of *Thymus algeriensis*, *Eucalyptus globulus* and *Rosmarinus officinalis* from Morocco. J Sci Food Agric. 2011 Nov;91(14):2643–51.
29. Jiang Y, Wu N, Fu YJ, Wang W, Luo M, Zhao CJ, Zu YG, Liu XL. Chemical composition and antimicrobial activity of the essential oil of *Rosemary*. Environ Toxicol Pharmacol. 2011 Jul;32(1):63–8.
30. Moumni S, Elaissi A, Trabelsi A, Merghni A, Chraief I, Jelassi B, Chemli R, Ferchichi S. Correlation between chemical composition and antibacterial activity of some Lamiaceae species essential oils from Tunisia. BMC Complement Med Ther. 2020 Apr 3;20(1):103.
31. Elamrani A, Zrira S, Benaissa M. Isolation of Moroccan *Rosmarinus eriocalix* Oil: Comparison Between Hydrodistillation and Microwave Extraction. J Essent Oil Bear Plants. 2003 Jan 1;6(1):1–8.
32. Chalchat JC, Gary RP, Michet A, Benjilali B, Chabart JL. Essential oils of *rosemary* (*Rosmarinus officinalis L*.). The chemical composition of oils of various origins (Morocco, Spain, France). J Essent Oil Res. 1993;5(6):613–8.
33. Malki F, Eddaraji K, Alloudane R, Greche H, Essalmani H, Barrijal S. Antimicrobial activity of essential oils extracted from leaves of native Moroccan plants against clinical bacterial isolates. Int Arab J Antimicrob Agents. 2018 Apr 9;8.
34. Kchikich A, Kirschvink N, El Kadili S, Raes M, El Otmani S, Bister JL, El Amiri B, Barrijal S, Chentouf M. *Thymus satureioides* and *Origanum majorana* essential oils improve the quality of Beni Arouss buck semen during storage at 4°C. Reprod Domest Anim Zuchthyg. 2021 Dec;56(12):1572–81.
35. Aimad A, Youness EA, Sanae R, El Moussaoui A, Bourhia M, Salamatullah AM, Alzahrani A, Alyahya HK, A Albadr N, Nafidi HA, Ouahmane L, Mohamed F. Chemical Composition and Antifungal, Insecticidal and Repellent Activity of Essential Oils From *Origanum compactum Benth*. Used in the Mediterranean Diet. Front Plant Sci. 2022;13:798259.
36. Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. A study of the minimum inhibitory concentration and mode of action of *oregano* essential oil, thymol and carvacrol. J Appl Microbiol. 2001 Sep;91(3):453–62.
37. Ultee A, Slump RA, Steging G, Smid EJ. Antimicrobial activity of carvacrol toward *Bacillus cereus* on rice. J Food Prot. 2000;63(5):620–4.
38. El Ouadi Y, Manssouri M, Bouyanzer A, Majidi L, Bendaif H, Elmsellem H, Shariati MA, Melhaoui A, Hammouti B. Essential oil composition and antifungal activity of *Melissa officinalis* originating from north-Est Morocco, against postharvest phytopathogenic fungi in apples. Microb Pathog. 2017 Jun;107:321–6.
39. Hu F, Tu XF, Thakur K, Hu F, Li XL, Zhang YS, Zhang JG, Wei ZJ. Comparison of antifungal activity of essential oils from different plants against three fungi. Food Chem Toxicol. 2019 Dec;134:110821.
40. Fliou J, Riffi O, Ali A, Elhourri M, Ghouati Y. Comparative study of the chemical composition of the essential oil of *origanum compactum* from the seven regions of morocco and their antimicrobial activity. J Microbiol Biotechnol Food Sci. 2020 Aug 2;10:7.
41. Hajlaoui H, Mighri H, Aouni M, Gharsallah N, Kadri A. Chemical composition and in vitro evaluation of antioxidant, antimicrobial, cytotoxicity and anti-acetylcholinesterase properties of Tunisian *Origanum majorana L*. essential oil. Microb Pathog. 2016 Jun;95:86–94.
42. Bouyahya A, Dakka N, Talbaoui A, Et-Touys A, El-Boury H, Abrini J, Bakri Y. Correlation between phenological changes, chemical composition and biological activities of the essential oil from Moroccan endemic Oregano (*Origanum compactum Benth*). Ind Crops Prod. 2017 Dec 1;108:729–37.
43. Lagrouh F, Dakka N, Bakri Y. The antifungal activity of Moroccan plants and the mechanism of action of secondary metabolites from plants. J Mycol Médicale. 2017 Sep 1;27(3):303–11.
44. El Khetabi A, Lahlali R, Askarne L, Ezrari S, El Ghadaroui L, Tahiri A, Hrustić J, Amiri S. Efficacy assessment of *pomegranate peel* aqueous extract for brown rot (*Monilinia spp*.) disease control. Physiol Mol Plant Pathol. 2020 Apr 1;110:101482.
45. Aourach M, Barbero GF, González de Peredo AV, Diakite A, El Boukari M, Essalmani H. Composition and antifungal effects of aqueous extracts of *Cymbopogon citratus*, *Laurus nobilis* and *Santolina chamaecyparissus* on the growth of Fusarium oxysporum f. sp. lentis. Arch Phytopathol Plant Prot. 2021 Dec 14;54(19–20):2141–59.
46. Kasmi M, Aourach M, El Boukari M, Barrijal S, Essalmani H. Efficacy of Aqueous Extracts of Aromatic and Medicinal Plants Against Gray Mold of Tomato in Morocco. C R Biol. 2017 Aug 1;340(8):386–93.
47. Moussa H, El Omari B, Chefchaou H, Tanghort M, Mzabi A, Chami N, Remmal A. Action of thymol, carvacrol and eugenol on *Penicillium* and *Geotrichum* isolates resistant to commercial

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

fungicides and causing postharvest citrus decay. Can J Plant Pathol. 2021 Jan 2;43(1):26–34.
48. Diánez F, Santos M, Parra C, Navarro MJ, Blanco R, Gea FJ. Screening of antifungal activity of 12 essential oils against eight pathogenic fungi of vegetables and mushroom. Lett Appl Microbiol. 2018 Oct;67(4):400–10.