Antifungal assessment, synergistic activity and molecular docking studies of essential oils as potential fungal inhibitor candidates against Alternaria alternata

Khaoula CHATTAT
National Institute of Agronomic Research: Institut National de la Recherche Agronomique
Rachid AZENZEM
National Institute of Agronomic Research: Institut National de la Recherche Agronomique
Amal Sammama
National Institute of Agronomic Research: Institut National de la Recherche Agronomique
Nada FANJAOUI
National Institute of Agronomic Research: Institut National de la Recherche Agronomique
Lahcen OUAHMANE
Cadi Ayyad University Faculty of Sciences Semlalia: Universite Cadi Ayyad Faculte des Sciences Semlalia
Tayeb KOUSSA
Chouaib Doukkali University Faculty of Sciences: Universite Chouaib Doukkali Faculte des Sciences
Mohamed Najib ALFEDDY
mohamednajib.alfeddy@inra.ma

National Institute for Agronomic Research https://orcid.org/0000-0002-5077-0113

Research Article

Keywords: Alternaria alternata, essential oils mixtures, synergism, ubiquitin E3 ligase, Molecular docking, Olea europea

Posted Date: June 6th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6679362/v1

Version of Record: A version of this preprint was published at European Journal of Plant Pathology on February 4th, 2026. See the published version at https://doi.org/10.1007/s10658-026-03186-6.

Antifungal assessment, synergistic activity and molecular docking studies of essential oils as potential fungal inhibitor candidates against *Alternaria alternata*

Khaoula Chattat [1,2] (0009-0007-2082-6124), Rachid Azenzem [1,2] (0000-0003-0666-3211), Amal Sammama [1] (0009-0004-4347-5724), Nada Fanjaoui [1,2] (0009-0008-5277-7299), Lahcen Ouahmane [3] (0000-0002-7378-549X), Tayeb Koussa [2] (0000-0002-9969-1678), Mohamed Najib Alfeddy [1] *(0000-0002-5077-0113)

[1] Plant Protection Research Unit, Regional Center of Agricultural Research of Marrakech, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco

[2] Laboratory of Plant Biotechnology, Ecology and Ecosystem Valorization, URL - CNRST N°10, Faculty of Sciences, Chouaïb Doukkali University, Av. Jabrane Khalil Jabrane, B.P. 299, 24000 El Jadida, Morocco

[3] Labeled Research Unit-CNRST N◦4, Laboratory of Microbial Biotechnology, Agro-Sciences and Environment (BioMAgE), Cadi Ayyad University, Marrakesh 40000, Morocco.

*Corresponding author: Mohamed Najib Alfeddy Email: mohamednajib.alfeddy@inra.ma

Abstract

The management of plant pathogens has become a challenge due to increasing pesticide resistance. Recent studies have focused on developing alternatives to chemical pesticides, enabling effective control of crop pathogens with reduced environmental impact and minimal effects on human health. This study examines the chemical composition and the antifungal activity of four essential oils extracted from *Origanum compactum*, *Thymus leptobotrys*, *Laurus nobilis,* and *Rosmarinus officinalis* in individual and combined forms against *Alternaria alternata* causal agent of olive leaf diseases. The most potent inhibitors of *A. alternata* were oregano and Thyme essential oils, distinguished by the predominance of carvacrol (59% and 75.05%), respectively. Furthermore, essential oil combinations such as oregano/thyme and oregano/thyme/laurel reduced the MIC values and showed synergistic effects (FICI≤0.5). *In silico* analysis by molecular docking was performed to study the anticipated ligands of these essential oils against Ubiquitin E3 Ligase responsible for H2B monoubiquitination in *Alternaria alternata*. These results suggest that, as part of the search for sustainable ways to reduce fungicide use in agriculture, essential oils, combinations, and even chemical compounds may be effective against *Alternaria alternata*.

Keywords: *Alternaria alternata*, essential oils mixtures, synergism, ubiquitin E3 ligase, Molecular docking, *Olea europea*

Introduction

The olive (*Olea europaea L.*) is a species of tree belonging to the family *Oleaceae*. It is one of the most characteristic trees of the Mediterranean basin and occupies a central position in the agricultural and environmental landscape (Reyes-Goya et al., 2024). However, climate change endangers their survival. It is causing problems and making trees more susceptible to pests and diseases. Changes in temperature and extreme weather conditions impact the incidence and appearance of diseases such as foliar diseases (Hamzaoui et al., 2025). Basım et al. (2017) reported that *A. alternata* is associated with foliar diseases of olive trees in Turkey and the prevalence of the disease has been estimated to be in the range of 40 and 80 %. In a study carried out in different nurseries to reveal the fungal species associated with olive trees in Morocco, *A. alternata* was found to be one of the most common fungal species isolated from olive leaves suspected of being infected, accounting for about 63% (Chliyeh et al., 2014). Several fungal species in the genus Alternaria are harmful to most cultivated plants and agricultural products. *Alternaria alternata* is known to cause leaf spots and diseases on many plant species, and postharvest rot on several types of fruit and vegetables (Schmey et al., 2024). Recently, using chemical antimicrobial agents has raised

concerns regarding environmental and human health impacts. Furthermore, the emergence of pesticide resistance is a new challenge for researchers in developing alternatives for various applications, including crop protection (Yılmaz et al., 2023). From this point of view, natural antimicrobial agents such as essential oils (EOs) have aroused growing interest in several sectors, with several studies highlighting their effectiveness (Ait Bouzid et al., 2024). Accordingly, the diversity of EOs and their chemical composition, as green products derived from aromatic and medicinal plants, have proven to be an innovative approach with remarkable efficacy in inhibiting the development of phytopathogenic fungi by inhibiting sporulation or by damaging cells, resulting in irreversible coagulation or denaturation of cellular components (Hou et al., 2022). Various computational approaches can be used to elucidate their mechanism of action. Molecular docking analysis remains one of the most important tools since it can predict the ability of a molecule to bind to the active site of a protein, providing an atomistic insight into molecular recognition. In this context, this study aims to evaluate the antifungal properties of EOs obtained from Oregano *(Origanum compactum),* Laurel *(Laurus nobilis),* Thyme *(Thymus leptobotrys),* and Rosemary *(Rosmarinus officinalis),* against *A. alternata,* causing fungal infections on various crops and agricultural products. It was also designed to study the chemical composition of these EOs and their synergistic effects on the growth of *A. alternata*. To confirm the results of the antifungal activity test, a molecular docking study against ubiquitin E3 ligase Bre1 was carried out.

Material and methods

Plant material

The aerial parts of *O. compactum* plants were collected in Chaouen, *R. officinalis, and Thymus leptobotrys* in the Marrakech region and Beni Mellal, respectively. Then, the leaves of *Laurus nobilis* were collected in Tafraout. Plant material was dried at room temperature (20-25°C) for one week.

EOs extraction

The EOs were extracted using the hydrodistillation method in a Clevenger-type apparatus. In detail, 150 g of dried material of each species was distilled in 1.5 L of water for 3 h. The essential oils obtained were stored at 4 °C in the dark until use. Extraction was carried out in three repetitions. EO yields were calculated (Razzouk et al., 2022). The combined effects of the four EOs were analyzed by preparing eleven mixtures.

Chemical analysis of EOs

The chemical composition of the extracted EOs was determined using gas chromatography-mass spectrometry (GC-MS). The GC-Ms was equipped with an Agilent DB-5 capillary column (stationary phase 5% phenyl/95% dimethyl siloxane, length 30 m, internal diameter 0.25 mm, and film thickness 0.1 µm). Analysis was performed with a column temperature program starting at 70°C and increasing at 3°C/min to 230°C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Injector and detector temperatures were maintained at 250°C. A volume of 1 µL of samples was injected for chromatography in fractionated mode (1:10) (Jeldi et al., 2022).

Fungal isolates

Alternaria alternata has been isolated from leaves of olive trees showing symptoms in the form of small spots located mainly on the margins of the upper leaf surface. Symptomatic olive leaves were collected in January and April 2023 in Hed Ras El Ain Rhamna, Marrakech. The isolates were identified by examining their morphological

characteristics (mycelium, conidiophores, and conidia) as described by previous researchers (Simmons, 1992; Woudenberg et al., 2013).

Pathogenicity test

Pathogenicity tests were carried out on healthy leaves of the Moroccan Picholine olive variety following the method described by (Basım et al., 2017) with modifications. Leaves were washed in tap water, disinfected in 70% ethanol for 30 seconds, and then washed in sterile water for one minute before being dried on sterile filter paper. Five olive leaves were placed on moist Whatman filter paper in the Petri dishes. The leaves were inoculated by placing a 6 mm diameter mycelial plug taken from the edges of a seven-day-old PDA culture of the isolates. The leaves were incubated at 25 ± 2°C in the dark. The in vivo test was performed on olive seedlings. Conidia were harvested from a 7-day culture with sterile distilled water and adjusted to 1×10^6 conidia/ml. Inoculation was performed by spraying the leaves with the prepared conidial suspension. Control plants were sprayed with water. After the incubation period, symptomatic samples were taken, and the suspected fungus was re-isolated and placed on PDA until Koch's postulates were confirmed.

Antifungal activity

Volatile activity assay (VA)

The technique adopted in this study is based on the vapor-phase method (Reyes-Jurado et al., 2020). It consists of culturing the microorganisms to be tested in Petri dishes on a PDA medium, and incubating these dishes in an inverted position. Different volumes (8µL, 16 µL, 32 µL, 64 µL/petri dish) of EOs were applied to a sterile filter paper placed in the center of the dish lid. The control was amended with the same volume of sterile distilled water, and three replicates were used per treatment. Plates were sealed with Parafilm® and incubated for 7 days at25 ± 2°C °C. Radial fungal growth was measured, and the percentage of inhibition was calculated.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The minimum inhibitory concentration (MIC) of essential oils was determined using the microdilution technique (Hänel and Raether, 1988). Fungal spore inoculum was prepared from 7-day-old cultures with 0.85% sterile saline. The spore suspension was adjusted to a concentration of approximately 2.3×10^4 spores/mL. The culture medium used was Tryptic Bile Soy Broth (TBS), incorporated with the fungal inoculum and the volumes of EOs studied. Inoculated tubes were incubated for 72 h at 25 ± 2°C. TBS medium alone was employed as a negative control, and inoculum without essential oils was used as a positive control.

The MIC was determined from the first tube showing a complete absence of mycelial growth. The minimum fungicidal concentration (MFC) was determined by sub-culturing 5 µL aliquots of the broth microdilution tube considered as MIC onto plates containing PDA medium.

Antifungal assays of mixtures

The potential synergistic, antagonistic, and indifferent activity of essential oils blends was studied using the broth microdilution method against *A. alternata*. The fractional inhibitory concentration index (FICI) was estimated using MIC values (Nikkhah and Hashemi 2020). The FIC index (FICi) was considered as: synergistic when FICi ≤ 0.5; indifferent effect when $0.5\leq$ FICi ≤ 2; and antagonistic when FICi ≥ 2 (Krisch et al., 2011).

Antifungal activity on detached olive leaves

The antifungal activity of EOs was evaluated on healthy olive leaves. They were treated with a 70% ethanol solution for 3 minutes and then rinsed with sterile distilled water. The leaves were sprayed with concentrations of essential oils corresponding to the MIC, then a 6 mm mycelial disc of 7-day-old *A. alternata* was placed on the

surface. Incubation was carried out at 25 ± 2°C in the dark for 8 days. The experiment was repeated three times, with three replicates per experiment. The inoculated control was sprayed with water and inoculated with *A. alternata*. A disc of culture medium was placed on the surface of the non-inoculated control. The severity of the disease (DS) and disease reduction (DR) on the inoculated leaves are evaluated at 7 days after infection by calculating the percentage of affected leaf surface. A visual scale from 0 to 5 based on disease symptoms was used: (1) no visible infection, (2) 5%, (3) 10%, (4) 20%, and (5) 50% or more of leaf area infected (Morkeliūnė et al., 2021).

Molecular docking

The protein-ligand interaction and affinity were determined by molecular docking. The ligand molecules were downloaded from PubChem, and then the online SMILES translator (https://cactus.nci.nih.gov/translate) was used to transform them into 3D structures in pdb format for highly efficient virtual screening (Adedotun et al., 2022). The crystal structure of Ubiquitin E3 Ligase synthase (PDB ID:7w76) was selected from the Protein Data Bank server (www.pdb.org). The protein active site has been determined by the CASTp server, which provides a map of the protein's topographic surface. AutoDockTools 1.5.7 was used to prepare the protein input files by removing all water molecules, polar hydrogens were added, and the prepared file was saved in pdbqt format. Thirty-four phytochemicals identified as major and minor compounds of the EOs studied were used in this study. Molecular docking analysis was performed by Pyrx-virtual screening tool (Open babel, Autodock Vina), based on the Auto Dock Vina configuration. The BIOVIA Discovery Studio tool (version 4.5) was used to determine the interactions between the protein-ligand complexes, where the various interactions were evaluated (Dey et al., 2023).

Statistical analysis

The results obtained are expressed as the mean ± standard deviation. The statistical analyses were performed using SPSS software (version 21.0). A one-way analysis of variance (ANOVA) with post-hoc Tukey was used to compare the results obtained. All treatments were performed in triplicate.

Results

Yield and chemical composition

The results showed that EO yields varied from 0.76±0.37% to 3.50±0.06%, GC-MS analysis reveals the chemical composition of the EOs. The results show that the EOs are distinguished by the diversity of their components, which belong to several families, with monoterpenes, phenols, and alcohols being the most abundant. The EOs of *O. compactum* and *T. leptobotrys* are characterized by carvacrol as the main compound. Whereas, the EOs of *L. nobilis* and *R. officinalis* are distinguished by the presence of 1,8-cineol (Table 1).

Table 1 The main chemical compounds detected in the essential oils used in this study

AMPs	Compounds	RI**	RT*(min)	Area (%)
Origanum compactum	**Carvacrol**	1292	12.485	**59**
	P-cymene	1023	7.167	**18.4**
	ϒ-terpinene	1057	7.852	**8.4**
	Carvacrol-methyl-ether	1244	11.595	2.8
	α -terpineol	1188	10.560	2.3
	Camphor	1146	9.661	2.1
	Camphene	951	5.725	1.8
	α-pinene	936	5.440	1.4
	α-terpinene	1018	7.852	1.3

	1,8-cineole	1029	7.321	0.9
	Linalool	1099	8.488	0.9
	Caryophyllene	1445	15.070	0.8
	Yield		3.50±0.06%	
Thymus leptobotrys	**Carvacrol**	1302	12.696	**75.05**
	γ-Terpinene	1057	7.849	**5.79**
	ρ-Cymene	1023	7.160	**5.71**
	Thymol	1292	12.483	2.46
	Camphor	1146	9.665	2.1
	Caryophyllene	1445	15.072	1.82
	α-Pinene	936	5.452	1.77
	Camphene	951	5.740	1.55
	Aromadendrene	1445	15.423	1.13
	α-Gurjunene	1407	16.408	1,12
	β-Bisabolene	1511	16.554	0.73
	Yield		1.71±0.07%	
Laurus nobilis	**1,8-cineole**	1029	7.323	**31.48**
	Camphene	951	5.740	**12.94**
	Linalool	1099	8.659	**12.13**
	Methyleugenol	1499	14.650	**9.16**
	α -terpineol	1188	–	**7.85**
	Eugenol	1358	13.819	**5.05**
	Sabinene	975	6.183	**4.08**
	2-Methyl-Z,Z-3,13-octadecadienol	1670	19.510	3.14
	Trans-isoeugenol	1568	18.909	1.88
	Bornylacetate	1279	12.467	1.85
	Trans-isoelemicin	1568	18.909	1.69
	α-pinene	936	5.740	1.66
	β-Elemene	1417	14.517	1.61
	β-Pinene	980	6.266	1.45
	Trans-cinnamylacetate	1448	15.398	1.37
	4-terpineol	1179	10.316	1.32
	Other			1.34
	yield		1.67±0.02%	
Rosmarinus officinalis	**1,8-Cineole**	1029	6.73	**33.46**
	Camphor	1146	7.67	**17.5**
	α-Pinene	936	5.72	**17.47**
	α-Terpinyl propionate	1468	8.01	7.63
	Borneol	1155	7.81	6.97
	m-Mentha-6,8-diene	1008	6.63	6.19
	Camphene	951	5.89	4.59
	Verbenone	1207	6.16	1.83
	Linalool	1099	8.25	1.52
	Terpinen-4-ol	1166	7.17	1.46

Bornyl acetate	1288	7.86	0.47
δ 3-carene	1058	8.87	0.16
Other			0.75
Yield		0.76±0.37%	

* Retention time, ** Retention index

Morphological Characterization, Isolation, and Pathogenicity Tests

The typical symptoms and morphology of *A. alternata* are showen in Figure 1 (A-F). The results show that the leaves of suspected infected olive trees have white spots on the adaxial surface with a diameter ranging from 2 to 7 mm. Fungal species are characterized by an aerated white-greyish mycelium with green inner zones on a PDA medium. *In vitro* pathogenicity tests in petri dishes did not show any spot symptoms, but dark brown lesions did develop on the surface of the leaves (Fig. 1 G). However, Pot-inoculated plants showed symptoms similar to those occurring in the field, although the size of the spots varied (Fig.1H).

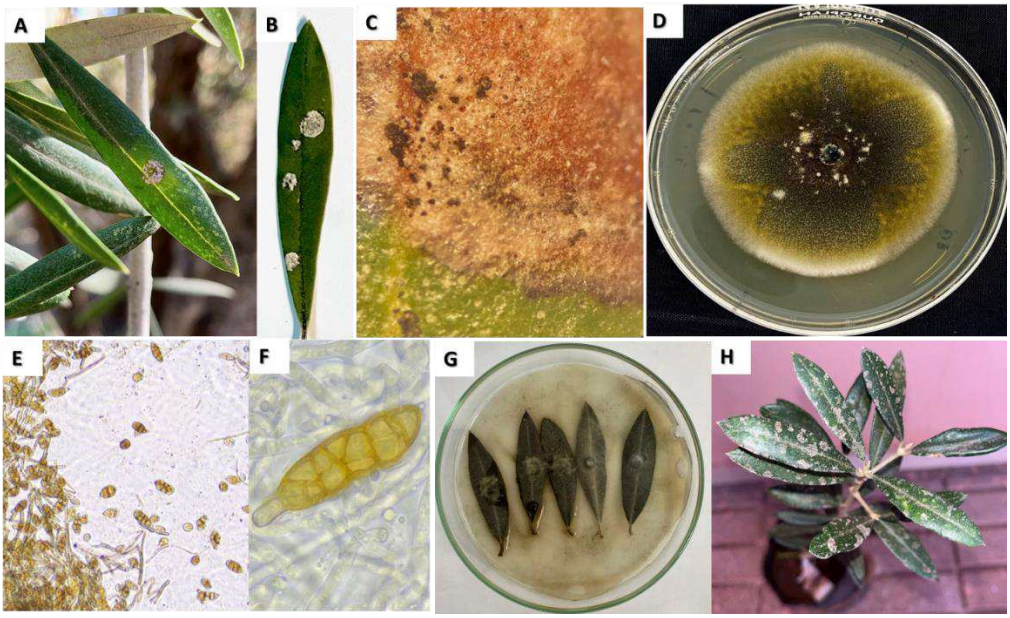


Fig. 1 The typical symptoms and morphological characteristics of *A. alternata*. **(A-C)** leaf spot symptoms, **D** Colony pattern on PDA, **(E-F)** Representative hyphae and conidia of *A. alternata,* (G-H) *In vivo* and *in vitro* pathogenicity tests

Antifungal activity

Determination of the diameter of mycelial growth zones

The antifungal activity of the EOs was evaluated by measuring the inhibition of mycelial growth of *A. alternata* at different concentrations compared to the negative control (Fig. 2). The results showed that all the EOs tested had an antifungal effect proportional to the concentration applied. The EOs of *T. leptobotrys* and *O. compactum* showed the best effects, with a total absence of radial mycelial growth from 8µL/petri dish. The diameter of mycelial growth varied from 50.17 to 34.67mm after application of the first two concentrations (8 and 16 µL/petri dish) of *L. nobilis* EO. In addition, EO of *R. officinalis* allowed complete growth inhibition only after application of the maximum concentration corresponding to 64 µL/petri dish (Fig. 3).

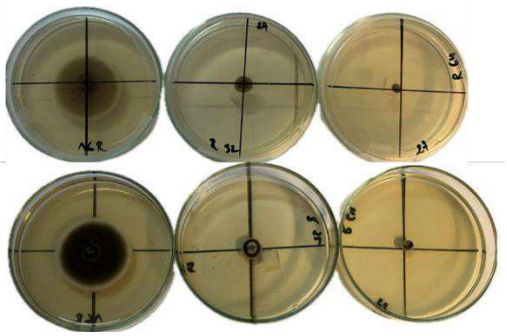


Fig. 2 *In vitro* antifungal activity.

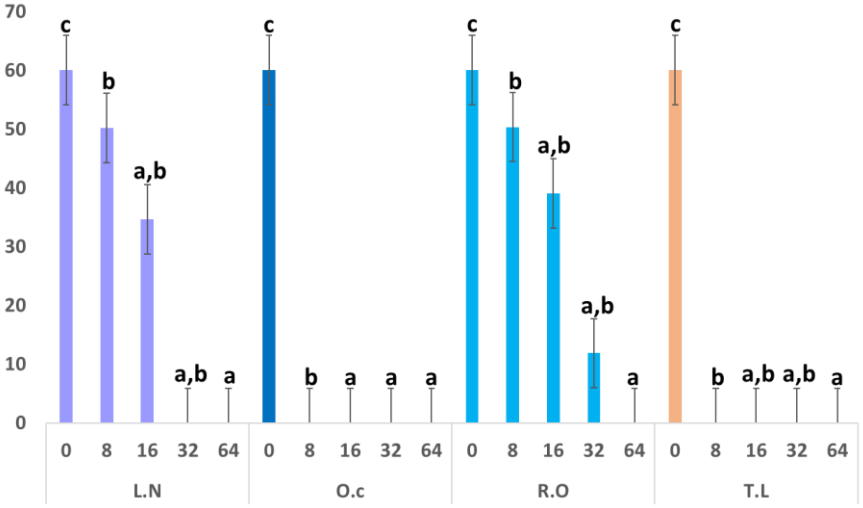


Fig. 3 Effect of the studied EOs on mycelial growth (mm) of *A. alternata*.

Determination of inhibition rate

According to the results presented in Figure 4, the EOs evaluated in this study exhibited different antifungal activities with a level of efficacy proportional to the concentrations used. *Origanum compactum* and *Thymus leptobotrys* EOs had the highest fungitoxicity effects against *A. alternata*, achieving 100% mycelial growth inhibition from a concentration of 8 µl/plate. Inhibition of *A. alternata* growth reached 100% at concentrations of 32µl/plate and 64µl/plate using *L. nobilis* and *R. officinalis* essential oils, respectively.

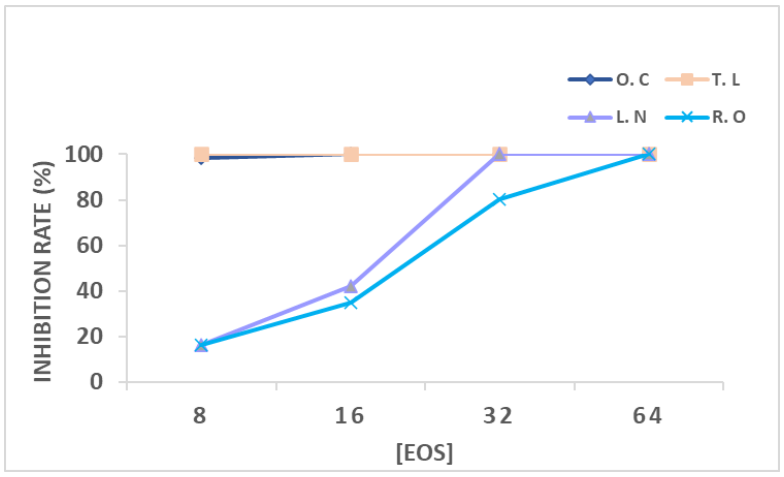


Fig. 4 Inhibition rate of EOs against *A. alternata*.

Determination of MIC and the synergistic effect of EOs

The results of the MIC and the MFC for the four studied EOs and their evaluated mixtures against *A. alternata* are presented in (Table 2). *O. compactum* EO exhibited the lowest MIC (0.650 µl/mL) compared to the other EOs tested.

Table 2 MIC and MFC values of EOs against *A. Alternata*

Essential oils	MIC * (µl/mL)	MFC ** (µl/mL)
O. compactum	0.651	0.651
T. leptobotrys	1.302	10.41
L. nobilis	5.206	10.41
R. officinals	10.41	20.83
Oregano/Thyme (M1)	0.16	0.16
Oregano/Laurel (M2)	2.60	10.41
Oregano/Rosemary (M3)	0.65	10.41
Thyme/Rosemary (M4)	2.60	20.825
Thyme/Laurel (M5)	1.302	5.206
Laurel/Rosemary (M6)	2.60	10.41
Oregano/Thyme/Laurel (M7)	0.16	1.302
Oregano/Thyme/Rosemary (M8)	0.325	5.206
Oregano/Rosemary/Laurel (M9)	0.325	5.206
Thyme/ / Rosemary/ Laurel (M10)	0.650	20.825
Oregano/Thyme/ Laurel/ Rosemary (M11)	0.325	5.206

(*) Minimal Inhibitory Concentration, (**) Minimal fungicidal concentration (n = 3) for each EO

The Oregano/Thyme, and Oregano/Thyme/Laurel mixtures showed very high inhibitory activity with a MIC of 0.16 µl/mL, compared to the Oregano/Thyme/Rosemary, Oregano/Rosemary/Laurel, and Oregano/Thyme/Laurel/Rosemary mixtures, which showed moderate inhibitory activity with a MIC of 0.325 µl/mL. In addition, *O. compactum* EO, Oregano/Thyme, and Oregano/Thyme/Laurel mixtures were also assessed as having high fungicidal activity with an MFC of 0.16, 0.65, and 1.302 µl/mL, respectively (Table 2).

furthermore, results revealed that the maximum synergistic effect was attributed to Origan-Thyme and Origan-Thyme-Laurier with a FICI of 0.37 and 0.4, respectively. Double Laurel-Rosemary combinations (FICI=0.75) had a partial synergistic effect. In contrast, Origano-Rosemary, Thyme-Rosemary, and Thyme-Laurier combinations showed an indifferent effect. The triple combinations Origano-Thyme-Rosemary (FICI=0.78), Origano-Rosemary-Laurier (FICI=0.59), and Thyme-Rosemary-Laurier (FICI=0.69) have presented partial synergism effect on *A. alternata*. The quadruple mixture of all the essential oils tested showed partial synergy (Table 3).

Table 3 Interaction activity of EO combinations

Combination of Eos	**FIC**		**FICI**	**Interpretation***
Double combination A/B	EO A	EO B		
Oregano/Thyme	0.25	0.12	0.37	Synergism
Oregano/Laurel	4.00	0.50	4.5	Antagonism
Oregano/Rosemary	1.00	0.06	1.06	Indifference
Thyme/Rosemary	2.00	0.25	2.25	Indifference

Thyme/Laurel	1.00	0.25			1.25	Indifference
Laurel/Rosemary	0.25	0.50			0.75	Partial synergism
Triple combination A/B/C	**FIC**				**FICI**	**Interpretation***
	EOA	EOB	EOC			
Oregano/Thyme/Laurel	0.25	0.03	0.12		0.40	Synergism
Oregano/Thyme/ Rosemary	0.50	0.03	0.25		0.78	Partial synergism
Oregano/Rosemary/Laurel	0.50	0.03	0.06		0.59	Partial synergism
Thyme/Rosemary/Laurel	0.06	0.12	0.50		0.69	Partial synergism
Quadruple combination A/B/C/D	**FIC**				**FICI**	**Interpretation***
	EOA	EOB	EOC	EOD		
Oregano/Thyme/Laurel/Rosemary	0.51	0.25	0.06	0.03	0.86	Partial synergism

Abbreviations: FIC: Fractional Inhibitory Concentration, FIC: Fractional inhibitory concentration. FICI: Fractional inhibitory concentration index

*Interpretation: Synergism (FICI ≤0.5), partial synergism (0.5 <FICI ≤1.0), indifference (1.0<FICI ≤4.0), and antagonism (FICI >4.0).

Determination of DS (%) and DR (%)

Antifungal activity on detached olive leaves was developed to determine the efficacy of essential oils against *A. alternata* (Fig. 5). The results showed that all the studied treatments, applied at a concentration equivalent to the MIC, reduced infection on the leaves compared with the inoculated control. The greatest percentage of disease reduction and the lowest degree of severity are obtained after applying combinations of EOs with synergistic effects (Table 4).

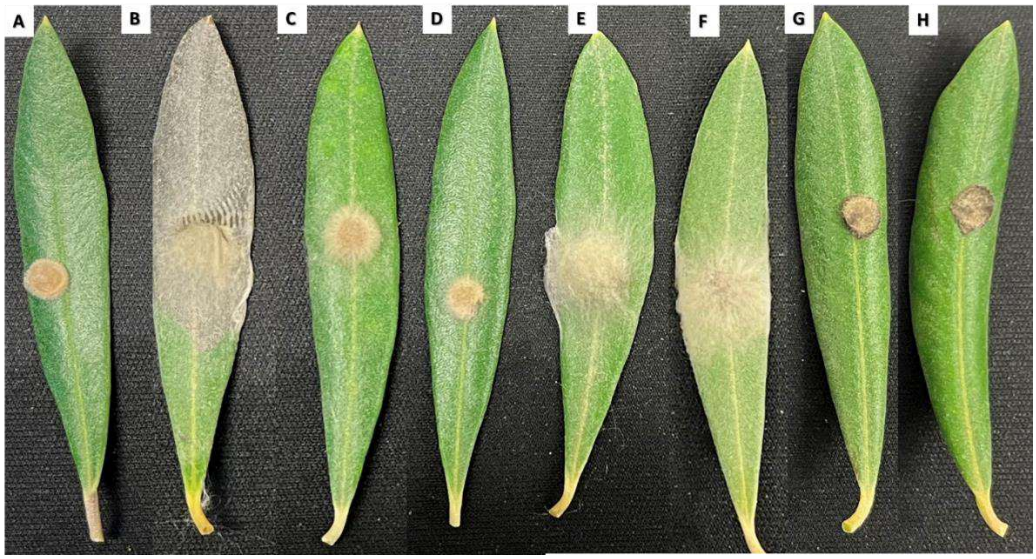


Fig. 5 Antifungal activity of EOs and their combination on detached olive leaves. (A) Cpntrol (B) Inoculated control, Inoculated and treated by (C) O.c, (D) T.L, (E) L.N, (F) RO, (G) Origano/Thyme, (H) Origano/Thyme/Laurel

Table 4 Disease severity and reduction of olive leaf spot by different essential oils 7 days after inoculation. Means n = 3 ± SE.

Treatments	Ds(%)	DR(%)
Inoculated control	86.6 ± 1.66	0
O.c	26.66 ± 1.66	73,72
T.L	22.77 ± 2.55	69,23
L.N	31.11 ± 0.96	64,1
RO	43.33 ± 1.66	50
Orig-Thy	21.66 ± 1.66	75
Orig-Thy-Lau	21.11 ± 0.96	75,64

Molecular docking study

Molecular docking assays were performed using a selected protein as a receptor and 32 plant metabolites as ligands. The analysis was preceded by preparing the protein and ligands and determining the active site. The predicted active binding site for the E3 ligase Bre1 protein is characterized by an active site surface area (ASA) of 5892.894 $Å^2$ and a volume (VSA) of 6632.236 $Å^3$ (Fig. 6).

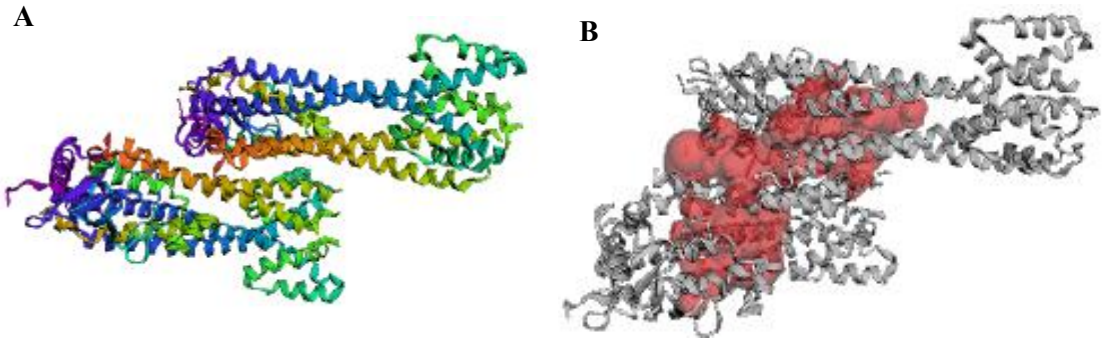


Fig. 6 3D representation of target protein (A) and predicted active site (B).

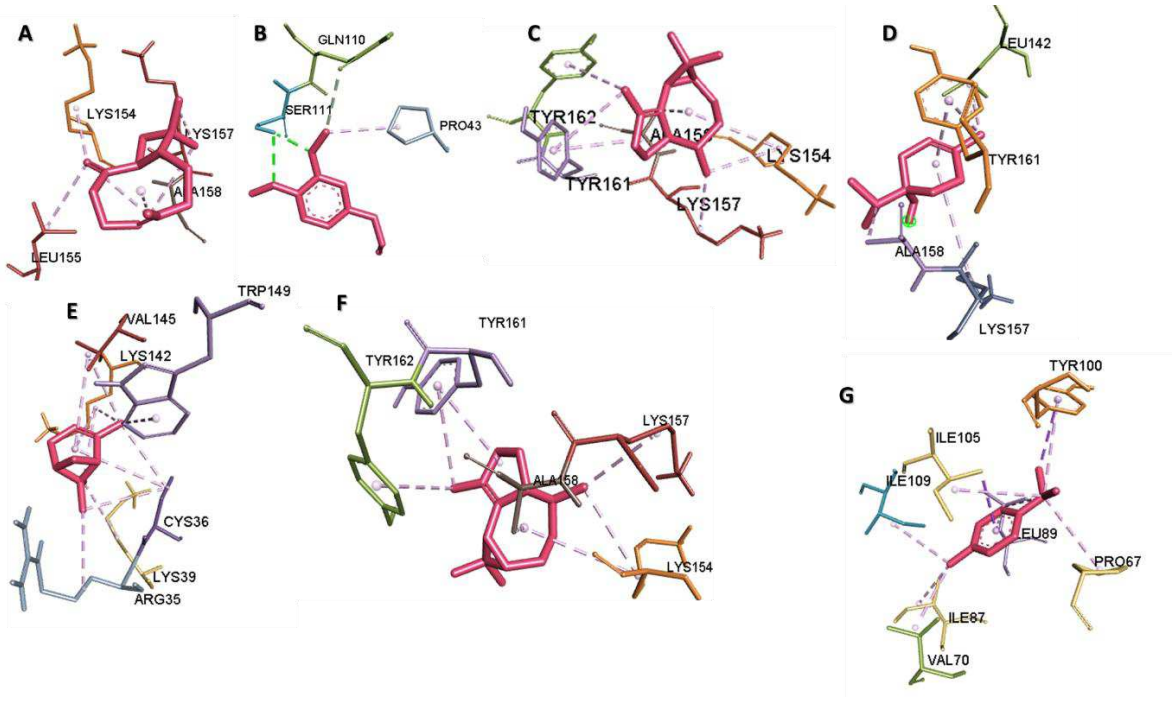
The results of these investigations, showing the estimated docking affinity of the docked positions, are presented in Table 5 and Figure 7. It was observed that all the compounds analyzed gave a better Gibbs free energy when interacting with the co-crystalline ligand of the target protein. Caryophyllene and Methyleugenol are the most stable of the other compounds, with the lowest Gibbs free energy (-9.7 and -9.3 kcal/mol, respectively). The binding sites, included LYS-154, LEU-155, ALA-157, ALA-158, and PRO-43, GLN-110, SER-111.In addition, Aromadendrene, 4-terpineol, α-gurjunene, α-pinene, γ-Terpinene, camphene, and camphor showed considerable stability in the active site, with Gibbs free energy ranging from -6.9 to -6 kcal/mol. The other components presented energies between -5.9 and -5.4 kcal/mol . The simulation revealed the interaction between the protein responsible for regulating hyphal growth, conidial formation, and pathogenicity of the *A. alternata* agent and the EOs chemical components, evaluated to explore the binding mechanisms of the selected protein to understand their role in inhibiting the pathogen.

Table 5 Binding sites of EOs components with target protein.

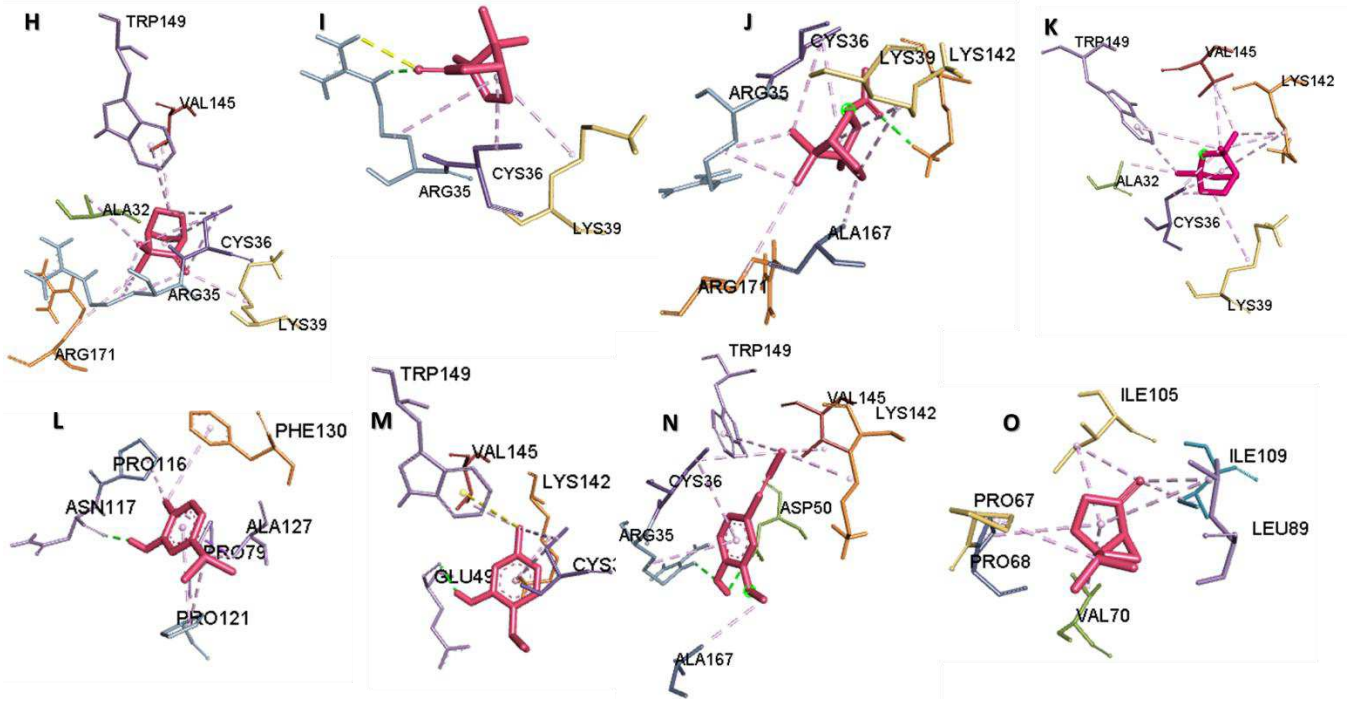
Ligands	Score (Kcal/mol)	Polar binding site
Caryophyllene	-9.7	LYS-154, LEU-155, ALA-157, ALA-158
Methyleugenol	-9.3	PRO-43, GLN-110, SER-111
Aromadendrene	-6.9	LEU-142, ALA-158, LYS-157, TYR-161
4-terpineol	-6.7	LEU-142, ALA-158, LYS-157, TYR-161
α-gurjunene	-6.5	LYS-154, Lys-157, ALA-158, TYR-161, TYR-162

α-pinene	-6.3	CYS-36, LYS-39, LYS-142, VAL-145, TRP-149
γ-Terpinene	-6	PRO67, VAL-70, ILE-87, LEU-89 TYR-100, ILE-100, ILE-109,
Camphene	-6	ALA-32, ARG-35, CYS-36, LYS-39, VAL-45, TRP-149, ARG-171
Camphor	-6	ARG-35, CYS-36, LYS-39
Bornyl acetate	-5.9	ARG-35, CYS-36, LYS-39, LYS-142, ALA-167, ARG-171
1,8-cineole	-5.7	ALA-32, CYS-36, LYS-39, LYS-142, VAL-145, TRP-149
Carvacrol	-5.7	PRO-79, PRO-116, ASN-117, PRO-121, ALA-127, PHE-130
Thymol	-5.7	CYS-36, GLU-49, LYS-142, VAL-145, TRP-149
Trans-isoeugenol	-5.7	ARG-35, CYS-36, ASP-50, LYS-142, VAL-145, TRP-149, ALA-167
Sabinene	-5.7	PRO-67, PRO-68, VAL-70, LEU-89, ILE-105, ILE-109
α -terpineol	-5.7	LYS-157, ALA-158, TYR-161, TYR-162
Trans-isoelemicin	-5.7	ALA-32, ARG-35, CYS-36, LYS-39, GLU-49, ASP-50, VAL-145, TRP-149, ALA-167 ARG-171
α-terpinene	-5.7	LYS-157, ALA-158, TYR-161, TYR-162
δ 3-carene	-5.7	LYS-157, ALA-158, TYR-161, TYR-162
p-Cymene	-5.6	LYS-157, ALA-158, TYR-161, TYR-162
β-Bisabolene	-5.6	PRO-67, VAL-70, ILE-87, LEU-89, TYR-100, ILE-105
Linalool	-5.6	ALA-32, ARG-35, CYS-36, LYS-39, LYS-42, VAL-145, TRP-149, ALA-167, ARG-171
Eugenol	-5.5	ARG-35, CYS-36, GLU-49, LYS-142, VAL-145, TRP-149, ALA-167
Verbenone	-5.5	ARG-35, CYS-36, LYS-39, LYS-142, TRP-149,ALA-167, ARG-171
α-Terpinyl propionate	-5.4	GLN-136, LEU-142, LYS-157, ALA-158, TYR-161, TYR-162
m-Mentha-6,8-diene	-5.3	ARG-35, CYS-36, LYS-39, LYS-142, VAL-145, TRP-149,ALA-167
β-Elemene	-5.3	LEU-142,LYS-157, ALA-158, TYR-161
Borneol	-5.3	ARG-35, CYS-36, LYS-39, GLU-49, LYS-142, VAL-145, TRP-149
β-Pinene	-5.1	ARG-35, CYS-36, LYS-39, LYS-142, VAL-145, TRP-149
Trans-cinnamylacetate	-5	LYS-157, ALA-158, TYR-161, TYR-162
2-Methyl-Z,Z-3,13octadecadienol	-4.8	LEU-13, PRO-16, LEU-155, THR-159, ARG-179
Carvacrol-methyl-ether	-4.4	LEU-142, ALA-158, LYS-157, TYR-161

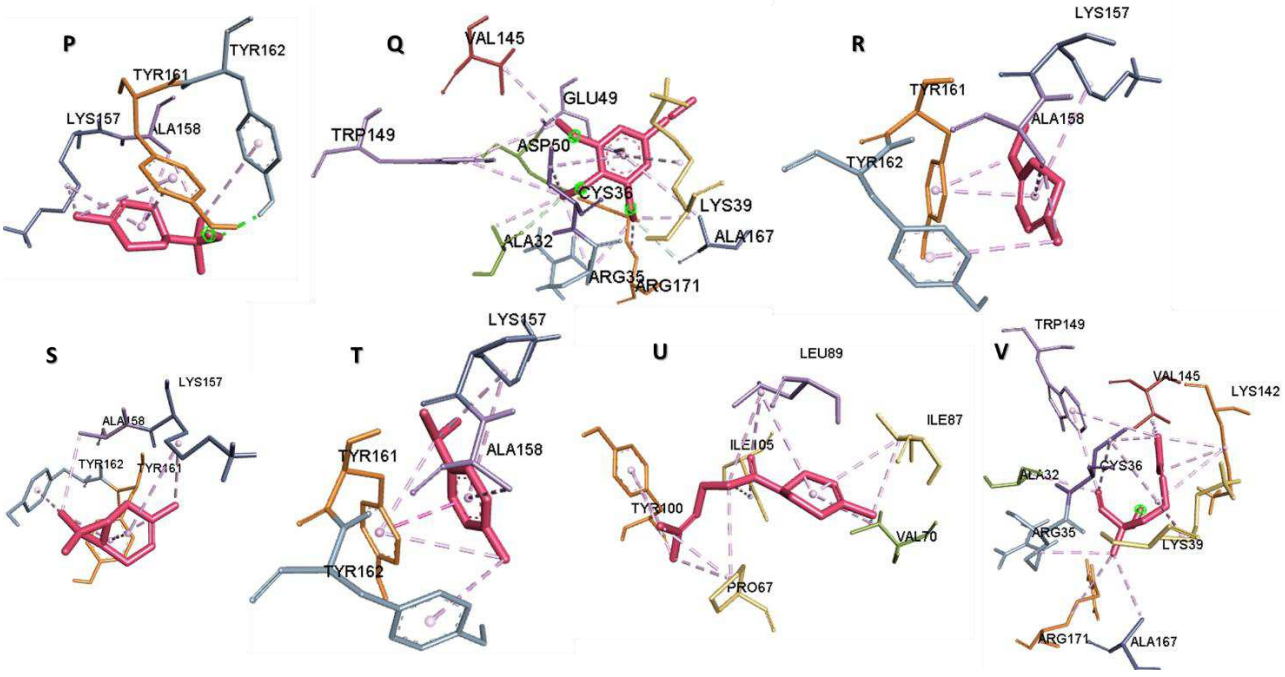
245


A
LYS154
LYS157
ALA158
LEU155
B
GLN110
SER111
PRO43
C
TYR162
TYR161
LYS154
LYS157
D
LEU142
TYR161
ALA158
LYS157
E
TRP149
VAL145
LYS142
CYS36
LYS39
ARG35
F
TYR161
TYR162
LYS157
ALA158
LYS154
G
TYR100
ILE105
ILE109
LEU89
PRO67
ILE87
VAL70

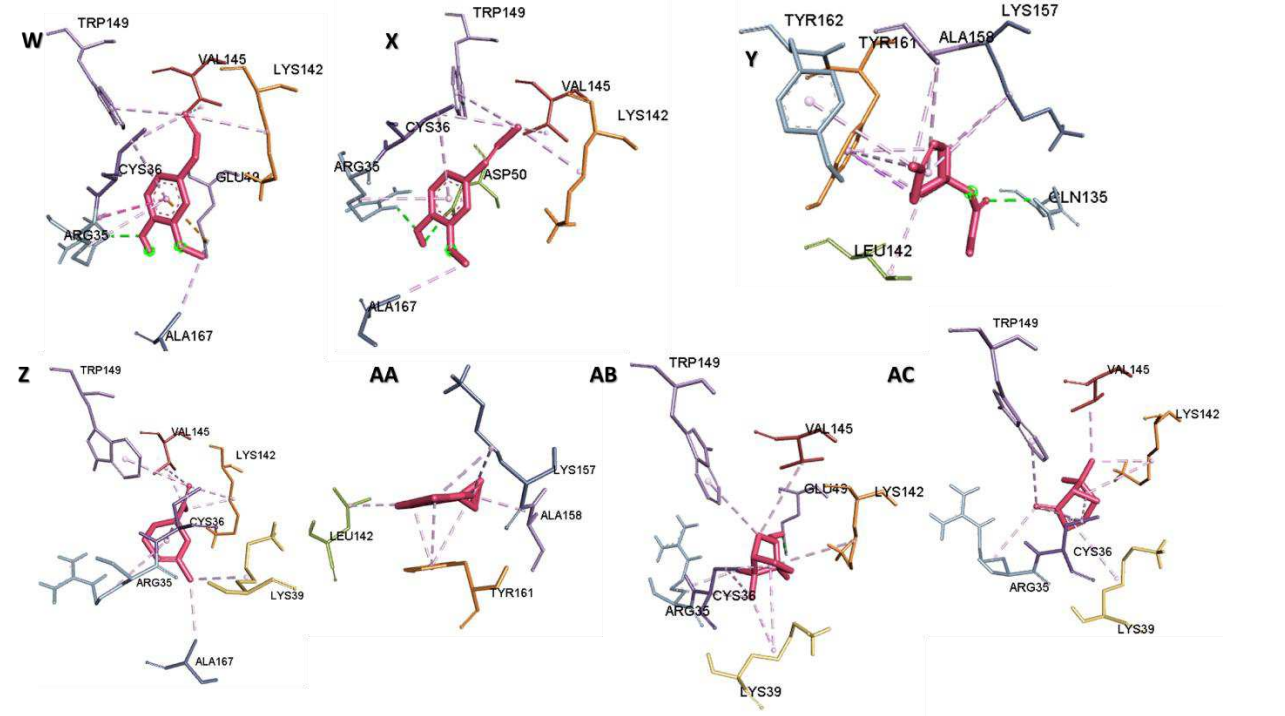
246


H
TRP149
VAL145
ALA32
CYS36
ARG35
LYS39
ARG171
I
ARG35
CYS36
LYS39
J
CYS36
LYS39
LYS142
ARG35
ALA167
ARG171
K
TRP149
VAL145
LYS142
ALA32
CYS36
LYS39
L
PHE130
PRO116
ASN117
ALA127
PRO79
PRO121
M
TRP149
VAL145
LYS142
GLU49
N
TRP149
VAL145
LYS142
CYS36
ASP50
ARG35
ALA167
O
ILE105
ILE109
PRO67
LEU89
PRO68
VAL70

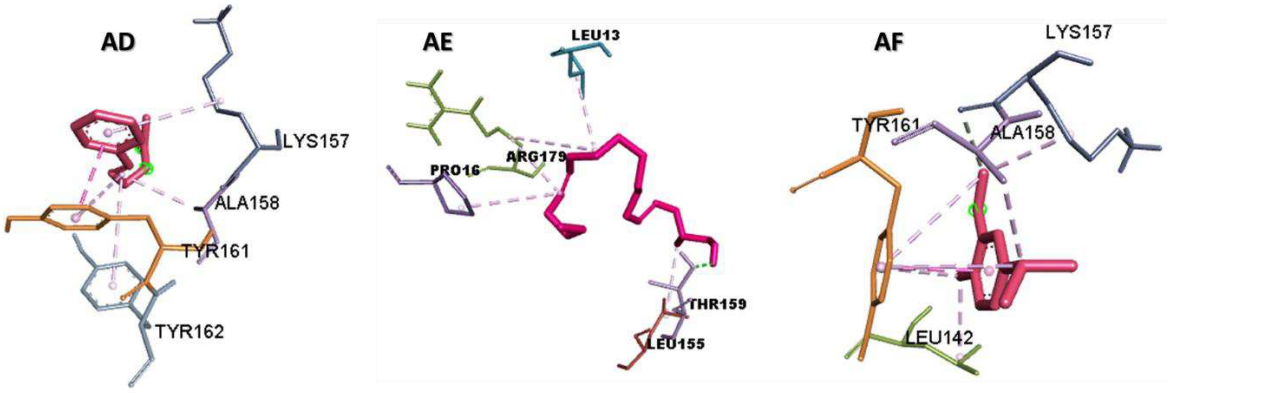
247


P
TYR162
TYR161
LYS157
ALA158
Q
VAL145
GLU49
TRP149
ASP50
CYS36
LYS39
ALA167
ALA32
ARG35
ARG171
R
LYS157
TYR161
ALA158
TYR162
S
LYS157
ALA158
TYR162
TYR161
T
LYS157
TYR161
ALA158
TYR162
U
LEU89
ILE87
ILE105
TYR100
VAL70
PRO67
V
TRP149
VAL145
LYS142
ALA32
CYS36
ARG35
LYS39
ARG171
ALA167

248


W
TRP149
VAL145
LYS142
CYS36
GLU49
ARG35
ALA167
X
TRP149
VAL145
LYS142
CYS36
ARG35
ASP50
ALA167
Y
TYR162
LYS157
TYR161
ALA158
GLN135
LEU142
Z
TRP149
VAL145
LYS142
CYS36
ARG35
LYS39
ALA167
AA
LYS157
ALA158
LEU142
TYR161
AB
TRP149
VAL145
GLU49
LYS142
CYS36
ARG35
LYS39
AC
TRP149
VAL145
LYS142
CYS36
ARG35
LYS39

249


AD
LYS157
ALA158
TYR161
TYR162
AE
LEU13
ARG179
PRO16
THR159
LEU155
AF
LYS157
TYR161
ALA158
LEU142

250

Fig. 7 Interaction of polar binding site residues in ubiquitin E3 Ligase.(A) Caryophyllene , (B) Methyleugenol, (C) Aromadendrene, (D) 4-terpineol, (E) α-gurjunene, (F) α-pinene, (G) γ-Terpinene, (H) Camphene, (I) Camphor; (G) Bornyl acetate, (K) 1,8-cineole, (L) Carvacrol, (M) Thymol, (N) Trans-isoeugenol, (O) Sabinene, (P) α-terpineol, (Q) Trans-isoelemicin, (R) α-terpinene, (S) δ 3-carene, (T) p-Cymene, (U) β-Bisabolene, (V) Linalool, (W) Eugenol, (X) Verbenone, (Y) α-Terpinyl propionate, (Z) m-Mentha-6,8-diene, (AA) β-Elemene, (AB) Borneol, (AC) β-Pinene, (AD) Trans-cinnamylacetate, (AE) 2-Methyl-Z,Z-3,13-octadecadienol, (AF) Carvacrol-methyl-ether

Discussion

Alternaria is a genus of phytopathogenic fungi that causes disease in many crops. It produces phytotoxins, which may explain its potential evolution and prevalence. *A. alternata* is an important and much-debated topic in current research due to its adaptation and acquisition of fungicide resistance (Bhushan et al., 2025). In this respect, this study was conceived to investigate the antifungal activities of EOS and their combinations against this plant pathogen. The yield and chemical composition of EOs can be impacted by many factors, including growing conditions, weather, altitude, soil type, agricultural practices, growth stage, part of the plant extracted, and harvesting season (Moghaddam & Mehdizadeh, 2017). In this sense, the EO yield (3.57±0.02%) obtained from *O. compactum* in this study is within the range (1.22% to 4.24%) reported by Laghmouchi et al. (2018) in different regions of northern Morocco. Furthermore, the chemical compounds (carvacrol and p-cymene) found in *O. compactum* EO are similar to those reported in several studies (Jeldi et al., 2022). *Thymus leptobotrys* essential oil yielded 1.71±0.07of EO in this investigation. Alaoui Jamali et al. (2014) obtained similar values for these species collected in the Tafrouat region of Morocco. The chemical composition of the essential oils obtained from *T. lebtobotrys* was mainly carvacrol (75.05%). Comparable results indicated that carvacrol (76.94-78.1%) is the predominant compound in the essential oil of *T. leptobotrys* (Etri & Pluhár, 2024). The yield of *Rosmarinus officinalis* EO was estimated at 0.77±0.38%, which is similar to the value found by Derwich et al. (2011) (0.54%) using aerial parts of these species collected in Boulemane province (Tafardoust). GC-MS analysis of *R. officinalis* essential oil identified 1,8-cineole (33.46%), followed by camphor (17.50%) and α-pinene (17.47%) as the main volatile compounds. This chemical composition is in accordance with that previously established for Moroccan Rosemary species (Elyemni et al., 2022). The yield of EO (1.66±0.02%) extracted from *L. nobilis* is similar to that obtained by Ailli et al. (2023) using the same extraction technique from plant material collected in the Meknes region. On the other hand, the prevalence of 1,8-cineol and linalool in the chemical composition of EO extracted from *L. nobilis* has been demonstrated in numerous studies, which point in the same direction as the present results (Razzouk et al., 2022).

Various levels of inhibition against this phytopathogenic agent have been demonstrated following the application of EOs extracted from aromatic and medicinal plants. This study highlighted the antifungal properties of *O. compactum*, *T. leptobotrys,* and their highest capacity to control *A. alternata*. These results are consistent with previous studies showing that the variation in fungicidal activity of plant EOs may be related to the diversity of active components (Hu et al., 2019). The findings of Ez-zriouli et al. (2020) highlighted that essential oils of *O. compactum* inhibited the growth of *A. alternata* at a MIC of 0.02%. Thyme has been shown to have a strong inhibitory effect on the fungus *A. alternata* in many studies (Ammar et al., 2022; Sazvar et al., 2022). Recent study showed that EOs and aqueous extracts of Thyme species such as *T. vulgaris*, *T. pectinatus*, and *T. convolutus* have potent antifungal properties on *A. alternata* (Francio et al., 2023). The antifungal properties of *L. nobilis* essential

oil have been studied in several studies (Fidan et al., 2019; Al-Abri et al., 2022). Ammar et al. (2022) examined the antifungal activity of EOs extracted from *L. nobilis* and *T. vulgaris* leaves. The in vitro test showed inhibition of radial mycelial growth of *A. alternata* ranging from 62 to 84% at concentrations of 2.5, 5, and 10%, respectively. *R. officinalis* EO had the lowest antifungal activity against *A. alternata* compared to the other EOs studied. Its effectiveness depends on the concentration used. Jalel et al. (2023) studied the activity of lemongrass and *R. officinalis* essential oils against *A. alternata,* showing that rosemary EO was less active against this fungus. For instance, Perina et al. (2015) evaluated *Thymus vulgaris* essential oil and thymol against *Alternaria alternata* and attributed the antifungal activity of this AMP to thymol. In another recent study, carvacrol was found to have the highest inhibitory activity against *A. alternata* when compared with 15 other components of essential oils (Li et al., 2023). In the current study, carvacrol is the main constituent of *O. compactum* and *T. leptobotrys*. These results underline the strong activity of these two essential oils against the causative agent.

The potential synergistic effect of combining EOs has been identified as an effective anti-microbial strategy (Bassolé & Juliani, 2012). There has been little research into the synergistic effect of EOs in inhibiting fungi, in comparison with the inhibition of the growth of bacteria and other pathogens (Nikkhah et al., 2017). The results of the present study demonstrated the ability of the chemical components of EOs to produce synergistic and partially synergistic effects on the inhibition of *A. alternata*. Hossain et al. (2016) demonstrated that the combination of Oregano and Thyme had a synergistic effect against numerous fungal pathogens of food (*Aspergillus flavus, Aspergillus parasiticus*, and *Penicillium chrysogenum*), due to the synergy between carvacrol and thymol. Nevertheless, Jiang et al. (2023) demonstrated that a mixture of Oregano and Thyme essential oils showed partial synergy against *A. alternata*. Further synergistic effects were reported using a combination of thyme and rosemary EOs on the growth of *A. alternata* (Nikkhah & Hashemi, 2020a). Complex interaction between different classes of compounds, such as phenols, aldehydes, alcohols, ketones, esters, ethers, or hydrocarbons present in EOs, often results in synergistic activity (Ngo-Mback et al., 2020). Therefore, the additive and synergistic activity of certain combinations against *A. alternata* demonstrated in this research is due to the combined effects of two or more EO components. Nevertheless, to our knowledge, no studies have yet been conducted on the antifungal effect of these EOs on detached olive leaves. This study demonstrates a positive effect in reducing the spread of *A. alternata* infection, with symptoms reducing by 75.64% and 50%. These results are significant, as they demonstrate that synergy enhances the efficacy of essential oils in vitro and on detached leaves.

A molecular docking study against ubiquitin E3 ligase was carried out to confirm the antifungal activity of the EOs components studied. This study was carried out to identify ligands potentially involved in blocking the enzyme's activity and thus to better understand their mode of action in controlling hyphal growth, conidiation, and pathogenicity of *A. alternata*. The major and minor components of the studied EOs bind to the target protein, as shown by the results of the *in silico* analysis. The most predicted ligands with high affinity to this protein are the natural chemical components Caryophyllene and Methyleugenol.

Ubiquitin E3 ligase is a key element in monoubiquitination (Fig. 8), and controls many vital fungal functions, including plant cell wall degradation enzymes and chitin-binding proteins, thought to be involved in hyphal growth, conidiation, and pathogenicity in *A. alternata* (Liu et al., 2020).

Numerous studies have explored the affinity of the main components of EOs towards various proteins, emphasizing their key role in observed biological activities (Adedotun et al., 2022; Omar et al., 2021). However, the results of the current study reveal that some minor components, despite being present in low concentrations,

may also exhibit a high affinity for protein targets. These results imply that the activity of EOs cannot be attributed exclusively to their major components. All constituents must be considered, including the minority fraction, as they can contribute significantly to the antifungal effect, notably through synergistic interactions between the different molecules present.

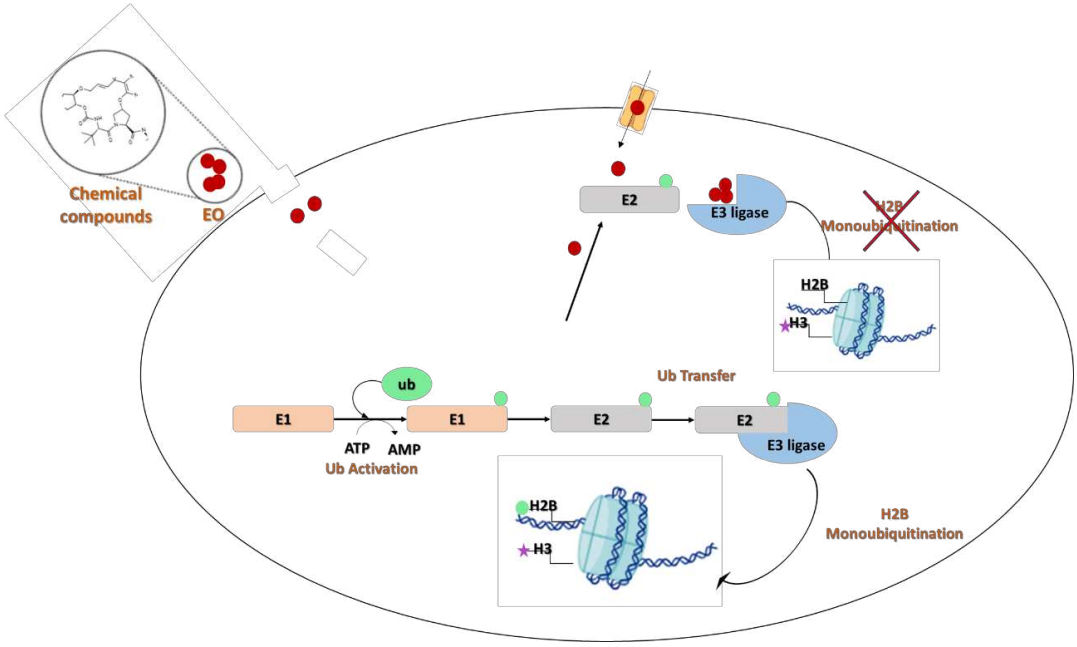


Fig. 8 Schema of the mechanism of action of essential oil on ubiquitin E3 ligase

Conclusion

In this investigation, the essential oils of *O. compactum* and *T. lebtobotrys* demonstrated the most potent antifungal activity against *Alternaria alternata,* causing leaf spots in olive leaves. The double (Oregano/Thyme) and triple (Oregano/Thyme/Laurel) combinations provided synergistic activity against *A. alternata*. This synergy highlights the interest in using associations of EOs to increase their efficacy in foliar disease control. These results provide a starting point for improving the efficacy of EOs by combining them. It also provides a theoretical basis and opens up new avenues for the use of EOs and their major as well as minor chemical compounds such us Caryophyllene and Methyleugenol as a new perspective in the context of ecological techniques aimed at limiting olive leaf spot caused by *A. alternata*, while minimizing the use of fungicides in agriculture.

Acknowledgments

The author Khaoula Chattat would like to thank the CNRST (Centre National pour la Recherche Scientifique et technique) in Morocco for the [Phd.-Associate Scholarship—PASS].

Author contributions

Alfeddy Mohamed Najib, Koussa Tayeb and Ouahmane Lahcen contributed to the study conception and design. Chattat Khaoula, Azenzem Rachid. performed material preparation, data collection and analysis. Chattat Khaoula wrote the first draft of the manuscript. Sammama Amal, Fanjaoui Nada, Alfeddy Mohamed Najib reviewed and edited the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by ongoing institutional funding. No additional grants to carry out or direct this particular research were obtained.

Declarations

Confict of interest and ethical approval The authors declare no conflict of interest. This research study complies with research and publishing ethics.

References

Adedotun, I. O., Abdul-Hammed, M., Hamzat, B. A., Adepoju, A. J., Akinboade, M. W., Afolabi, T. I., & Ismail, U. T. (2022). Molecular docking, ADMET analysis, and bioactivity studies of phytochemicals from Phyllanthus niruri as potential inhibitors of hepatitis C virus NSB5 polymerase. *Journal of the Indian Chemical Society*, *99*(2), 100321. https://doi.org/10.1016/j.jics.2021.100321

Ailli, A., Handaq, N., Touijer, H., Gourich, A. A., Drioiche, A., Zibouh, K., Eddamsyry, B., El Makhoukhi, F., Mouradi, A., Bin Jardan, Y. A., Bourhia, M., Elomri, A., & Zair, T. (2023). Phytochemistry and biological activities of essential oils from six aromatic medicinal plants with cosmetic properties. *Antibiotics*, *12*(4), 721. https://doi.org/10.3390/antibiotics12040721

Ait Bouzid, H., Ibourki, M., Hamdouch, A., Oubannin, S., Asbbane, A., Hallouch, O., Bijla, L., Koubachi, J., Majourhat, K., & Gharby, S. (2024). Moroccan aromatic and medicinal plants: a review of economy, ethnobotany, chemical composition, and biological activities of commonly used plants. *Food and Humanity*, *2*(January), 100259. https://doi.org/10.1016/j.foohum.2024.100259

Aksit, H., Bayar, Y., Simsek, S., & Ulutas, Y. (2022). Chemical composition and antifungal activities of the essential oils of Thymus species (Thymus pectinatus, Thymus convolutus, Thymus vulgaris) against plant pathogens. *Journal of Essential Oil-Bearing Plants*, *25*(1), 200–207. https://doi.org/10.1080/0972060X.2022.2043189

Alaoui Jamali, C., Kasrati, A., Bekkouche, K., Hassani, L., Wohlmuth, H., Leach, D., & Abbad, A. (2014). Cultivation and the application of inorganic fertilizer modifies essential oil composition in two Moroccan species of Thymus. *Industrial Crops and Products*, *62*, 113–118. https://doi.org/10.1016/j.indcrop.2014.08.017

Ammar, H. A., El-Helaly, S. H., & Abdel-Rahman, T. A. (2022). Efficacy of some plant extracts and fungicides to control alternaria black spots caused by Alternaria alternata on postharvest mango fruits in Egypt. *Menoufia Journal of Plant Protection*, *7*(3), 29–40. https://doi.org/10.21608/mjapam.2022.228715

Basım, E., Basım, H., Abdulai, M., Baki, D., & Öztürk, N. (2017). Identification and characterization of Alternaria alternata causing leaf spot of olive tree (Olea europaea) in Turkey. *Crop Protection*, *92*, 79–88. https://doi.org/10.1016/j.cropro.2016.10.013

Bassolé, I. H. N., & Juliani, H. R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules*, *17*(4), 3989–4006. https://doi.org/10.3390/molecules17043989

Bhushan, B. T., Alam, S. H., Mahapatra, S., Chakraborty, S., & Hooi, A. (2025). Alternaria. *Compendium of Phytopathogenic Microbes in Agro-Ecology* , 1–21. https://doi.org/10.1007/978-3-031-81770-0_1

Chliyeh, M., Rhimini, Y., Selmaoui, K., Touhami, A. O., Filali-Maltouf, A., El Modafar, C., Moukhli, A., Oukabli, A., Benkirane, R., & Douira, A. (2014). Survey of the fungal species associated to olive-tree

(Olea europaea L.) in Morocco. *International Journal of Recent Biotechnology*, *2*(2), 15–32.

Derwich, E., Benziane, Z., Chabir, R., & Taouil, R. (2011). In vitro antibacterial activity and GC/MS analysis of the essential oil extract of leaves of Rosmarinus officinalis grown in Morocco. *International Journal of Pharmacy and Pharmaceutical Science*, *3*(3), 89–95.

Dey, D., Hossain, R., Biswas, P., Paul, P., Islam, M. A., Ema, T. I., Gain, B. K., Hasan, M. M., Bibi, S., Islam, M. T., Rahman, M. A., & Kim, B. (2023). Amentoflavone derivatives significantly act towards the main protease (3CLPRO/MPRO) of SARS-CoV-2: in silico admet profiling, molecular docking, molecular dynamics simulation, network pharmacology. *Molecular Diversity*, *27*(2), 857–871. https://doi.org/10.1007/s11030-022-10459-9

Elyemni, M., El Ouadrhiri, F., Lahkimi, A., Elkamli, T., Bouia, A., & Eloutassi, N. (2022). Chemical composition and antimicrobial activity of essential oil of wild and cultivated Rosmarinus officinalis from two Moroccan localities. *Journal of Ecological Engineering*, *23*(3), 214–222. https://doi.org/10.12911/22998993/145458

Etri, K., & Pluhár, Z. (2024). Exploring chemical variability in the essential oils of the Thymus genus. *Plants*, *13*, 1375. https://doi.org/https://doi.org/10.3390/ plants13101375

Ez-zriouli, R., Yacoubi, H. El, Oubihi, A., & Zahra, F. (2020). Study of the antifungal activity of Origanum compactum essential oil against different plant pathogenic fungi. *Plant Archives*, *20*(2), 5331–5336.

Francio, I. E., Paulus, D., Lermen Munhoz, C., & Luckmann Balbinotti de França, D. (2023). Chemical composition of five essential oils and their antioxidant and in vitro and in vivo antifungal activities against Alternaria alternata in tomato crop. *Natural Product Research*, 1–9. https://doi.org/10.1080/14786419.2023.2285879

Hamzaoui, H., Maafa, I., Choukri, H., Bakkali, A. El, Houssaini, S. E. I. El, Razouk, R., Aziz, A., Louahlia, S., & Habbadi, K. (2025). Assessment of Peacock Spot Disease (Fusicladium oleagineum) in Olive Orchards Through Agronomic Approaches and UAV-Based Multispectral Imaging. *Horticulturae*, *11*(1), 1–18. https://doi.org/10.3390/horticulturae11010046

Hänel, H., & Raether, W. (1988). A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. *Mycoses*, *31*(3), 148–154.

Hossain, F., Follett, P., Dang Vu, K., Harich, M., Salmieri, S., & Lacroix, M. (2016). Evidence for synergistic activity of plant-derived essential oils against fungal pathogens of food. *Food Microbiology*, *53*, 24–30. https://doi.org/10.1016/j.fm.2015.08.006

Hou, T., Sana, S. S., Li, H., Xing, Y., Nanda, A., Netala, V. R., & Zhang, Z. (2022). Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience*, *47*(November 2021), 101716. https://doi.org/10.1016/j.fbio.2022.101716

Hu, F., Tu, X. F., Thakur, K., Hu, F., Li, X. L., Zhang, Y. S., Zhang, J. G., & Wei, Z. J. (2019). Comparison of antifungal activity of essential oils from different plants against three fungi. *Food and Chemical*

Toxicology, *134*(September), 110821. https://doi.org/10.1016/j.fct.2019.110821

Jalel, S. J., Abed, I. J., & Jasim, A. N. (2023). Evaluating the biological activity of lemongrass and rosemary essential oils against some fungi isolated from vegetables and fruits. *Journal of Population Therapeutics & Clinical Pharmacology*, *30*(April), 46–54. https://doi.org/10.47750/jptcp.2023.30.08.005

Jeldi, L., Taarabt, K. O., Mazri, M. A., Ouahmane, L., & Alfeddy, M. N. (2022). Chemical composition, antifungal and antioxidant activities of wild and cultivated Origanum compactum essential oils from the municipality of Chaoun, Morocco. *South African Journal of Botany*, *147*, 852–858. https://doi.org/10.1016/j.sajb.2022.03.034

Jiang, S., Wang, L., Li, D., & Sun, C. (2023). The purification of dominant spoilage fungi on Lonicera Caeruleum and the inhibitory effects of composite essential oils against these fungi. *Food Bioscience*, *53*(May), 102839. https://doi.org/10.1016/j.fbio.2023.102839

Krisch, J., Tserennadmid, R., & Vágvölgyi, C. (2011). Essential oils against yeasts and moulds causing food spoilage. *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*, *November 2014*, 1135–1142.

Laghmouchi, Y., Belmehdi, O., Senhaji, N. S., & Abrini, J. (2018). Chemical composition and antibacterial activity of Origanum compactum Benth. essential oils from different areas at northern Morocco. *South African Journal of Botany*, *115*, 120–125. https://doi.org/10.1016/j.sajb.2018.02.002

Li, H., Ding, J., Liu, C., Huang, P., Yang, Y., Jin, Z., & Qin, W. (2023). Carvacrol treatment reduces decay and maintains the postharvest quality of red grape fruits (Vitis vinifera L.) inoculated with Alternaria alternata. *Foods*, *12*(23), 4305. https://doi.org/10.3390/foods12234305

Liu, Y., Xin, J., Liu, L., Song, A., Liao, Y., Guan, Z., Fang, W., & Chen, F. (2020). Ubiquitin E3 ligase aabre1 responsible for H2B monoubiquitination is involved in hyphal growth, conidiation and pathogenicity in alternaria alternata. *Genes*, *11*(2), 1–15. https://doi.org/10.3390/genes11020229

Moghaddam, M., & Mehdizadeh, L. (2017). Chemistry of essential oils and factors influencing their constituents. In A. M. G. and A. M. Holban (Ed.), *Soft Chemistry and Food Fermentation* (pp. 379–419). Academic Press. https://doi.org/10.1016/B978-0-12-811412-4/00013-8

Morkeliūnė, A., Rasiukevičiūtė, N., Šernaitė, L., & Valiuškaitė, A. (2021). The use of essential oils from thyme, sage and peppermint against colletotrichum acutatum. *Plants*, *10*(1), 1–11. https://doi.org/10.3390/plants10010114

Ngo-Mback, M. N. L., Babii, C., Jazet Dongmo, P. M., Kouipou Toghueo, M. R., Stefan, M., & Fekam Boyom, F. (2020). Anticandidal and synergistic effect of essential oil fractions from three aromatic plants used in Cameroon. *Journal de Mycologie Medicale*, *30*(2), 100940. https://doi.org/10.1016/j.mycmed.2020.100940

Nikkhah, M., & Hashemi, M. (2020a). Boosting antifungal effect of essential oils using combination approach as an efficient strategy to control postharvest spoilage and preserving the jujube fruit quality. *Postharvest*

Biology and Technology, *164*(July 2019), 111159. https://doi.org/10.1016/j.postharvbio.2020.111159

Nikkhah, M., & Hashemi, M. (2020b). Postharvest biology and technology boosting antifungal effect of essential oils using combination approach as an efficient strategy to control postharvest spoilage and preserving the jujube fruit quality. *Postharvest Biology and Technology*, *164*(March), 111159. https://doi.org/10.1016/j.postharvbio.2020.111159

Nikkhah, M., Hashemi, M., Habibi, M. B., & Farhoosh, R. (2017). Synergistic effects of some essential oils against fungal spoilage on pear fruit. *International Journal of Food Microbiology*, *257*(March), 285–294. https://doi.org/10.1016/j.ijfoodmicro.2017.06.021

Omar, H. S., Abd El-Rahman, S. N., AlGhannam, S. M., Reyad, N. E. H. A., & Sedeek, M. S. (2021). Antifungal evaluation and molecular docking studies of olea europaea leaf extract, thymus vulgaris and boswellia carteri essential oil as prospective fungal inhibitor candidates. *Molecules*, *26*(20). https://doi.org/10.3390/molecules26206118

Perina, F. J., Amaral, D. C., Fernandes, R. S., Labory, C. R. G., Teixeira, G. A., & Alves, E. (2015). Thymus vulgaris essential oil and thymol against Alternaria alternata (Fr.) Keissler: Effects on growth, viability, early infection and cellular mode of action. *Pest Management Science*, *71*(10), 1371–1378. https://doi.org/10.1002/ps.3933

Raho Ghalem, B., Talia, B., & Omar, H. (2020). Antifungal activities of five commercial extracts against Alternaria alternate. *Journal of Biotechnology Research*, *6*(8), 98–103. https://doi.org/10.32861/jbr.68.98.103

Razzouk, S., Mazri, M. A., Jeldi, L., Mnasri, B., Ouahmane, L., & Alfeddy, M. N. (2022). Chemical composition and antimicrobial activity of essential oils from three mediterranean plants against eighteen pathogenic bacteria and fungi. *Pharmaceutics*, *14*(8). https://doi.org/10.3390/pharmaceutics14081608

Reyes-Goya, C., Santana-Garrido, Espinosa-Martín, P., Vázquez, C. M., & Mate, A. (2024). Wild and cultivated olive trees: Nutraceutical insights of extra virgin olive oils in cardiovascular and ocular diseases. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1870*(1). https://doi.org/10.1016/j.bbadis.2023.166904

Reyes-Jurado, F., Navarro-Cruz, A. R., Ochoa-Velasco, C. E., Palou, E., López-Malo, A., & Ávila-Sosa, R. (2020). Essential oils in vapor phase as alternative antimicrobials: a review. *Critical Reviews in Food Science and Nutrition*, *60*(10), 1641–1650. https://doi.org/10.1080/10408398.2019.1586641

Schmey, T., Tominello-Ramirez, C. S., Brune, C., & Stam, R. (2024). Alternaria diseases on potato and tomato. *Molecular Plant Pathology*, *25*(3), 1–19. https://doi.org/10.1111/mpp.13435

Simmons, E. . (1992). Alternaria taxonomy : current status, viewpoint, challenge. In C. and A. Visconti (Ed.), *Alternaria Biology, Plant Diseases and Metabolites* (pp. 1–35). Elsevier Science Publishers. https://cir.nii.ac.jp/crid/1570572699585565952

Woudenberg, J. H. C., Groenewald, J. Z., Binder, M., & Crous, P. W. (2013). Alternaria redefined. *Studies in*

Mycology, *75*, 171–212. https://doi.org/10.3114/sim0015

Xie, Y., Wang, Z., Huang, Q., & Zhang, D. (2017). Antifungal activity of several essential oils and major components against wood-rot fungi. *Industrial Crops & Products*, *108*(June), 278–285. https://doi.org/10.1016/j.indcrop.2017.06.041

Yılmaz, G. E., Göktürk, I., Ovezova, M., Yılmaz, F., Kılıç, S., & Denizli, A. (2023). Antimicrobial nanomaterials: a review. *Hygiene*, *3*(3), 269–290. https://doi.org/10.3390/hygiene3030020

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Highlights1.docx