Original article Casp. J. Vet. Sci., 2026; 3(1): 65-78

Amol University of Special Modern Technologies

Caspian Journal of Veterinary Sciences

doi: 10.22034/cjvs.2026.573309.1058

Journal homepage: https://cjvs.ausmt.ac.ir/

Origanum vulgare ssp. *viridulum* and ssp. *vulgare* essential oils against agronomically critical fungal and bacterial pathogens

Seyed Ali Andi[1], Mahmoud Kiani[1*], Mahboobeh Zare[1], Fereshteh Maskani[1]

[1] Faculty of Medicinal Plants, Amol University of Special Modern Technologies, Amol, Iran.

(*) Corresponding Author: m.kiani@ausmt.ac.ir

Article Info

Article history:

Submit Date: 2 February 2026

Accept Date: 3 May 2026

Online Date: 17 June 2026

Keywords:

Essential oil profiling

Oregano

Phytopathogen biocontrol

Subspecific differentiation

Thymol chemotype

Abstract

Fungal and bacterial phytopathogens cause substantial agricultural losses, motivating development of natural antimicrobial alternatives to synthetic pesticides. This study evaluated the antimicrobial efficacy of essential oils (EOs) from two *Origanum vulgare* subspecies, ssp. *viridulum* (Behshahr, Iran) and ssp. *vulgare* (Chalus, Iran), against five fungal and five gram-negative bacterial phytopathogens. GC-MS profiling delineated two chemotypes: a thymol chemotype (ssp. *viridulum*: thymol 29.9%, γ-terpinene 13.0%, β-pinene 11.3%; 0.40% v/w yield) and a linalyl acetate chemotype (ssp. *vulgare*: linalyl acetate 27.2%, γ-terpinene 16.5%, 3-octanone 10.9%; 0.10% v/w yield). Factorial bioassays (disc and well diffusion, three concentrations) demonstrated that ssp. *viridulum* exhibited superior overall efficacy (50.07% mean inhibition) compared to ssp. *vulgare* (30.22%). Strong fungal selectivity was observed (64.49% mean inhibition) relative to bacterial pathogens (15.75%), a 4.1-fold difference attributable to the Gram-negative outer membrane permeability barrier. *Sclerotinia sclerotiorum* was the most susceptible pathogen (90.24% mean inhibition; 100% at 5 µl), while *Pseudomonas syringae* was the least responsive (12.81%). These results established ssp. *viridulum* EO as a candidate natural antifungal for post-harvest and protected-cultivation applications, contingent on *in vivo* and formulation validation. Bacterial disease management requires complementary strategies. The findings linked subspecies-level chemotypic variation to differential biocontrol efficacy, supporting chemotype-based EO standardization for targeted crop protection.

Introduction

Fungal and bacterial phytopathogens account for 20–40% of direct production losses in major agricultural crops globally, with annual economic costs estimated in the hundreds of billions of dollars (Ayaz *et al*., 2023; Wang *et al*., 2024). Fungal pathogens, in particular, represent the most economically diverse and damaging class, responsible for annual crop losses estimated between 10% and 20%, which translates to a financial burden of $100–200 billion worldwide each year, encompassing highly destructive diseases like white mold (*Sclerotinia sclerotiorum*) and mycotoxin producers (*Fusarium* spp. and *Aspergillus* spp.) (Godfray *et al*., 2016; Wang *et al*., 2024). Bacterial phytopathogens, though structurally distinct, also exact substantial economic tolls, with specific diseases, such as fire blight (*Erwinia amylovora*) and citrus canker (*Xanthomonas citri*) causing multi-million or even billion-dollar losses annually (Wang *et al*., 2024). Traditional disease management has relied predominantly on synthetic fungicides and bactericides, which, despite their efficacy, have generated escalating concerns regarding environmental contamination, non-target organism toxicity, human health risks from pesticide residues, and the accelerating emergence of pathogen populations resistant to commonly deployed active ingredients (Ayaz *et al*., 2023). These concerns necessitate an urgent and comprehensive paradigm shift toward ecologically benign, yet highly effective, alternatives for integrated disease management.

Essential oils (EOs), complex extracts derived from aromatic plants, have garnered considerable scientific and industrial attention as promising natural antimicrobial agents capable of supplementing or replacing synthetic options. These natural products inherently possess advantages crucial for sustainable agriculture, including a multi-component chemistry that typically acts upon multiple cellular targets simultaneously, thereby presenting a significantly lower risk profile for the emergence of pathogen resistance compared to single-target synthetic molecules (Yap *et al*., 2014; Chouhan *et al*., 2017; Kesraoui *et al*., 2022). Among the vast array of potential plant-derived candidates, oregano (*Origanum vulgare* L.) stands out as a preeminent source, supported by a long-standing history of use in traditional medicine and food preservation throughout Mediterranean and Middle Eastern cultures (Leyva-López *et al*., 2017). The remarkable antimicrobial efficacy of oregano EO is primarily attributed to its phenolic monoterpenes, particularly thymol and its structural isomer carvacrol (Andi and Maskani, 2021; Zamuner *et al*., 2023). Furthermore, *Origanum* EO offers significant practical benefits vital for large-scale adoption, including its Generally Recognized as Safe (GRAS) status, making it highly compatible with organic farming practices and post-harvest food safety applications. These favorable regulatory and safety characteristics, combined with dramatically reduced environmental persistence, position *Origanum* EO as a highly attractive foundation for next-generation crop protection strategies.

Despite the established reputation and substantial commercial interest in *O. vulgare*, its large-scale deployment remains impeded by a critical knowledge gap stemming from extreme intraspecific chemical variability. The precise chemical profile of *O. vulgare* is highly plastic and dynamically influenced by a complex interplay of genetic factors (subspecies or chemotype), geographical origin, local environmental conditions, and specific cultivation and postharvest practices. This inherent plasticity results in significant chemical divergence between populations (Andi and Maskani, 2021). While comparative analyses of *O. vulgare* chemotypes have been reported, including population-level surveys of Iranian *viridulum* accessions (Andi and Maskani, 2021) and intraspecific divergence studies under abiotic stress (Azimzadeh *et al*., 2023), a critical gap persists: the direct, paired evaluation of subspecies-level chemotypes against a broad, taxonomically diverse panel of economically important fungal and bacterial phytopathogens under identical experimental conditions. The inability to consistently correlate specific chemotypes with quantifiable biocontrol efficacy across multiple pathogen classes hampers chemotype-based standardization and constrains rational selection of oregano populations for targeted disease management. To directly address this crucial knowledge deficit, the present study performed a rigorous, integrated evaluation focused on two genetically and biogeographically distinct *O. vulgare* subspecies indigenous to northern Iran. The two populations selected for investigation were *O. vulgare* ssp. *viridulum* and *O. vulgare* ssp. *vulgare*, collected from the low-elevation coastal region of Behshahr and the

elevated terrain near Chalus, respectively. We hypothesized that the differing ecological pressures of these distinct habitats would drive an adaptive specialization resulting in divergent chemical phenotypes. Therefore, the primary aim of this research was twofold: first, to definitively characterize the complete EO chemical profiles of both native Iranian subspecies using established gas chromatography-mass spectrometry (GC-MS); and second, to systematically quantify and compare the antimicrobial efficacy of the EOs from both chemically divergent chemotypes against a carefully selected panel of ten major phytopathogens. This panel included five fungal species (*S. sclerotiorum, Magnaporthe salvinii, Fusarium verticillioides, Rhizoctonia solani,* and *Aspergillus flavus*) and five gram-negative bacterial species (*X. citri, Pectobacterium carotovorum, Agrobacterium tumefaciens, Erwinia amylovora,* and *Pseudomonas syringae*), which collectively represent major threats to diverse economically important crops. This study links subspecies-specific chemistry with antimicrobial efficacy to enable chemotype-based standardization of *Origanum* EOs for targeted crop protection.

Materials and Methods

Plant materials

The aerial parts of *O. vulgare* ssp. *viridulum* and ssp. *vulgare* were gathered in summer 2023 from adjacent but chemically distinct populations on the southern Caspian littoral (Fig. 1). The ssp. *viridulum* accession originated from open, grazed grassland on the eastern outskirts of Behshahr city (36° 41′ 37″ N 53° 33′ 11″ E; ≈ 25 m a.s.l.), whereas ssp. *vulgare* was collected from rocky, sun-exposed shelves in the lower Chalus River gorge immediately south of Chalus city (36° 39′ 17″ N 51° 25′ 16″ E; ≈ 25 m a.s.l.) where Route 59 begins its climb into the Alborz. Despite their ~222 km road-distance separation along the southern Caspian shore, both stations share a warm-temperate, humid Caspian climate: mean annual temperature ≈ 18°C (January mean ≈ 9°C; July mean ≈ 27°C), 830–865 mm precipitation falling mainly October–March, and year-round relative humidity of 77–79 %.

Botanical authentication was performed by Prof. Vahideh Nazeri, University of Tehran. Voucher specimens (No. 6391) for both populations were deposited in the Herbarium of the Horticultural Department, Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Fig. 1. Map of the collection localities in northern Iran. Plant materials for ssp. *viridulum* and ssp. *vulgare* were sampled from Behshahr (Eastern Mazandaran) and Chalus (Western Mazandaran), respectively.

EO isolation

The aerial parts designated for EO extraction were air-dried at room temperature under dark conditions, and then ground into a fine, homogeneous powder immediately before EO extraction to ensure optimal yield and compositional integrity. EOs were obtained by hydrodistillation for 3 hours at 100°C using an all-glass Clevenger-type apparatus, following the procedure outlined in the British Pharmacopoeia 1980 (Paterson, 1982). Oil yield was calculated as milliliters per 100 g of dry plant material. The oils were dried over anhydrous sodium sulfate, and stored in amber vials at 4°C until analysis.

EO profiling

Gas chromatography (GC) was performed on a Thermoquest instrument equipped with a flame ionization detector (FID) (Varian CP 3800, Japan) and fitted with a fused silica capillary DB-5 column (60 m × 0.25 mm i.d., 0.25 µm film thickness). Injector and detector temperatures were set at 250°C and 300°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.1 ml/minute. The oven program was: 60°C initial temperature, increased at 4°C/minute to 250°C, and held isothermally at 250°C for 10 minutes. The split ratio was 1:50.

Gas chromatography–mass spectrometry (GC-MS) was carried out using a Themoquest-Finnigan gas chromatograph coupled to a TRACE mass spectrometer (Manchester, UK) equipped with the

same DB-5 column. Helium served as the carrier gas. Electron impact ionization was performed at 70 eV, with ion source and interface temperatures of 200°C and 250°C, respectively. Mass spectra were acquired over a range of 43–456 amu. The GC-MS oven program was identical to that used for GC-FID.

The constituents of the EOs were identified by calculating their retention indices (RI) relative to a homologous series of *n*-alkanes (C_8–C_{24}) analyzed under identical temperature-programmed conditions on a DB-5 capillary column. Compound identification was achieved by comparing the obtained mass spectra with those stored in the Adams and Wiley 7.0 mass spectral libraries and by co-chromatography with authentic reference compounds. Further confirmation was made by comparing the experimentally determined RI values with those of authentic standards or with literature data (Adams, 2017). Quantification of individual components was based on relative peak area percentages obtained from GC–FID chromatograms without applying correction factors.

Preparation of pathogenic strains

The bacterial pathogens (*X. citri*, *A. tumefaciens*, *E. amylovora*, *P. carotovorum*, and *P. syringae*) were obtained from the Iranian Research Institute of Plant Protection. The fungal pathogens were supplied by the Department of Plant Pathology, Faculty of Agriculture, University of Tehran, Tehran, Iran. These fungal isolates originated from diverse agricultural sources: *F. verticillioides* and *M. salvinii* from infected rice plants, *R. solani* from potato crops, *S. sclerotiorum* from diseased sunflower plants, and *A. flavus* from contaminated pistachio samples.

For bacterial culture media, 20 g of nutrient agar (NA) powder was dissolved in 1 litre of distilled water, sterilized by autoclaving at 121°C for 20 minutes, and poured into sterile 9 cm Petri dishes. For fungal cultures, 39 g of potato dextrose agar (PDA; potato extract, dextrose, and agar) was dissolved in 1 litre of distilled water, sterilized under the same conditions, and dispensed into sterile 9 cm Petri dishes.

Antimicrobial activity

The antibacterial activity of the EOs was evaluated using the well diffusion method. Bacterial suspensions were adjusted to 10^8 CFU/ml, and 100 µl of each suspension was evenly spread onto NA plates. Wells (5 mm diameter) were aseptically punched into the agar, and 2, 5, and 8 µl of EO were introduced into the wells. Plates were incubated at 27°C for 48 hours, after which the diameters of the inhibition zones were measured (Valgas *et al*., 2007).

The antifungal activity was determined using the paper disc diffusion method. Fungal strains were cultured on PDA at 25°C, and 5 mm mycelial discs were excised from the actively growing margins of colonies. These discs were transferred onto fresh PDA plates. Sterile filter paper discs impregnated with 2, 5, or 8 µl of EO were placed on the agar surface, and the plates were sealed with Parafilm and incubated at 25°C (Magaldi *et al*., 2004). Colony growth was monitored, and reductions in diameter were compared to untreated controls. The percentage of growth inhibition was calculated using the formula:

$$I=\frac{C-T}{C}\times 100$$

Where I represented growth inhibition percentage, C represented the colony diameter of the control (untreated), and T represented the colony diameter of the treated group.

Statistical analysis

The experiment was conducted as a factorial arrangement within a completely randomized design (CRD), incorporating EO type, concentration, and pathogen as treatment factors, with three replicates per treatment (each replicate consisting of a single Petri dish). Data were analyzed using MSTAT-C software, and mean comparisons were performed using Duncan's multiple range test at $p < 0.05$.

Results and discussion

EO profiling

GC-MS analysis of EOs from both subspecies revealed distinct chemical compositions with notable quantitative and qualitative differences (Table 1). Data analysis identified 26 compounds in ssp. *viridulum* (Behshahr) and 19 compounds in ssp. *vulgare* (Chalus), with identifications accounting for 98.9% and 99.0% of the total EOs, respectively. The EO yield was markedly higher in

ssp. *viridulum* at 0.40% (v/w) compared to 0.10% (v/w) in ssp. *vulgare*, representing a 4-fold difference in oil production.

Both subspecies shared a conserved monoterpene hydrocarbon foundation, comprising 40.6% in ssp. *viridulum* and 42.3% in ssp. *vulgare*. Oxygenated monoterpenes represented 39.4% in ssp. *viridulum* and 36.4% in ssp. *vulgare*. Sesquiterpene hydrocarbons were presented at 2.9% and 3.5%, respectively. Oxygenated sesquiterpenes were detected exclusively in ssp. *viridulum*, constituting 1.0% of the EO, while completely absent in ssp. *vulgare*, indicating subspecies-level differentiation in sesquiterpene oxidation capacity. The remaining compounds (aldehydes, ketones, alcohols, and esters) comprised 15.0% in ssp. *viridulum* and 16.8% in ssp. *vulgare*. The greater overall compound richness in ssp. *viridulum* (26 vs. 19 total compounds) was accompanied by higher sesquiterpene diversity: 7 sesquiterpenes in ssp. *viridulum* versus 3 in ssp. *vulgare*.

The ssp. *viridulum* profile was dominated by a single apex compound: thymol at 29.9%, representing nearly one-third of the total EO. The remaining major constituents (>10%) were γ-terpinene (13.0%) and β-pinene (11.3%), together with thymol accounting for 54.2% of the oil. Moderate-level compounds (5-10%) included 3-octanone (9.2%), carvacrol (5.2%), and sabinene (5.0%), bringing the cumulative contribution of the top five compounds to 73.6%. Minor compounds (<5%) encompassed α-pinene (4.5%), 1-octen-3-yl acetate (3.2%), (E)-β-ocimene (2.8%), 1,8-cineole (2.0%), linalool (1.7%), (E)-2-hexenal (1.7%), myrcene (1.5%), α-thujene (1.5%), α-cadinene (1.4%), (E,E)-α-farnesene (1.1%), 1-octen-3-ol (0.9%), nerol (0.6%), camphene (0.5%), (E)-nerolidol (0.5%), caryophyllene oxide (0.5%), α-terpinene (0.5%), α-humulene (0.4%), and traces of geranyl acetate, trans-caryophyllene, and β-sesquiphellandrene.

The ssp. *vulgare* profile exhibited more distributed apex architecture. linalyl acetate dominated at 27.2%, followed by γ-terpinene (16.5%) and 3-octanone (10.9%), together representing 54.6% of the oil. Moderate-level compounds (5-10%) were limited to β-pinene (8.4%) and carvacrol (6.4%), bringing the top five compounds to 69.4%. The remainder consisted of minor compounds (<5%): α-terpinene (4.7%), α-pinene (3.4%), sabinene (3.2%), 1,8-cineole (2.8%), 1-octen-3-yl acetate (2.6%), camphene (1.9%), 1-octen-3-ol (1.9%), (E)-β-ocimene (1.6%), trans-caryophyllene (1.4%), (E)-2-hexenal (1.4%), α-thujene (1.3%), myrcene (1.3%), bicyclogermacrene (1.1%), and (E)-β-farnesene (1.0%).

Shared major and moderate constituents exhibited notable concentration divergences in ssp. *viridulum* vs. ssp. *vulgare*: β-pinene (11.3% vs. 8.4%), γ-terpinene (13.0% vs. 16.5%), 3-octanone (9.2% vs. 10.9%), sabinene (5.0% vs. 3.2%), and carvacrol (5.2% vs. 6.4%). Among shared minor compounds, α-terpinene showed the most dramatic difference: 0.5% in ssp. *viridulum* versus 4.7% in ssp. *vulgare*, representing a 9.4-fold elevation in ssp. *vulgare*. Other shared compounds with divergent levels included 1,8-cineole (2.0% vs. 2.8%), (E)-β-ocimene (2.8% vs. 1.6%), 1-octen-3-yl acetate (3.2% vs. 2.6%), and (E)-2-hexenal (1.7% vs. 1.4%).

Subspecies-specific compounds revealed the fundamental chemical differentiation: ssp. *viridulum* uniquely accumulated thymol (29.9%), linalool (1.7%), nerol (0.6%), and a suite of sesquiterpenes and sesquiterpene oxides: α-humulene (0.4%), (E, E)-α-farnesene (1.1%), α-cadinene (1.4%), (E)-nerolidol (0.5%), caryophyllene oxide (0.5%), β-sesquiphellandrene (trace), and geranyl acetate (trace). Conversely, ssp. *vulgare* uniquely produced linalyl acetate (27.2%), and bicyclogermacrene (1.1%). The linalool, present in ssp. *viridulum* at 1.7%, was entirely absent in ssp. *vulgare*, indicating this precursor alcohol is converted to linalyl acetate rather than accumulating as a free form. Notably, oxygenated sesquiterpenes were presented exclusively in ssp. *viridulum* (1.0%) and entirely absent in ssp. *vulgare*, indicating subspecies-level differentiation in sesquiterpene oxidation capacity.

The distinct chemical profiles observed in this study delineated two well-defined chemotypes within *O. vulgare*, reflecting intraspecific variation in secondary metabolite composition. ssp. *viridulum*, sourced from Behshahr, represented a thymol chemotype characterized by thymol (29.9%), carvacrol (5.2%), and oxygenated sesquiterpenes (1.0%), dominating the EO composition. In contrast, ssp. *vulgare*, from Chalus, represented a linalyl acetate chemotype, with linalyl acetate (27.2%), 3-octanone (10.9%), and no detectable oxygenated sesquiterpenes. The

apparent mutual exclusivity of thymol in ssp. *vulgare* and linalyl acetate in ssp. *viridulum* (both below GC-MS detection limits) suggests distinct biosynthetic pathways, with these compounds serving as diagnostic markers for each chemotype. This near-mutual exclusivity, combined with multiple diagnostic compounds, is consistent with divergence at downstream biosynthetic branch points; however, whether this reflects heritable genetic fixation of the relevant enzymatic steps or environmentally induced phenotypic plasticity cannot be determined from field-collected single-accession material. Common-garden experiments or comparative transcriptomic analyses of terpene synthase and cytochrome P450 gene families across multiple accessions of each subspecies would be required to partition genetic from environmental contributions to this chemotypic divergence. The phenolic-rich profile of ssp. *viridulum* suggests potentially superior antimicrobial activity compared to ssp. *vulgare*.

EO biosynthesis proceeds from isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), C_5 precursors generated through the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways (Rehman *et al*., 2016; Hou *et al*., 2020), which are sequentially condensed by prenyltransferases to form geranyl pyrophosphate (GPP, C_{10}) and farnesyl pyrophosphate (FPP, C_{15}) (Hou *et al*., 2020; Lange *et al*., 2000). Terpene synthases then cyclize these intermediates into monoterpene hydrocarbons (40.6% in ssp. *Viridulum* and 42.3% in ssp. *vulgare*) and sesquiterpene hydrocarbons (2.9% and 3.5%, respectively) (Lange *et al*., 2000; Rehman *et al*., 2016; ; Escobar *et al*., 2020; de Sousa *et al*., 2023). The near-identical monoterpene hydrocarbon (40.6% vs. 42.3%) and sesquiterpene hydrocarbon (2.9% vs. 3.5%) fractions across subspecies reveal a conserved early biosynthetic infrastructure: both subspecies possess equivalent terpene synthase capacity and commit similarly to hydrocarbon skeleton formation (Crocoll *et al*., 2010; Azimzadeh *et al*., 2023). The critical divergence emerges at downstream oxygenation branch points, where the two subspecies commit to fundamentally different enzymatic investments. Ssp. *viridulum* prioritizes cytochrome P450–mediated monooxygenase activity, directing p-cymene hydroxylation preferentially toward thymol (29.9%) over the isomeric carvacrol (5.2%), a 5.8:1 ratio indicating regulatory prioritization (Crocoll, 2011; Krause *et al*., 2021; Hao *et al*., 2022;). Additionally, ssp. *viridulum* synthesizes oxygenated sesquiterpenes (1.0%) through sesquiterpene oxidase activity, demonstrating broad monooxygenase engagement (Agliassa and Maffei, 2018). In contrast, ssp. *vulgare* suppresses phenolic biosynthesis (carvacrol only 6.4%, thymol absent) and instead prioritizes acetyl-transferase activity, rapidly converting terpene alcohols to acetate esters: linalool (1.7% in ssp. *viridulum*) is completely absent in ssp. *vulgare*, having been acetylated to linalyl acetate (27.2%) (Larkov *et al*., 2008; Zaks *et al*., 2008).

The two populations are geographically separated by approximately 220 km along the southern Caspian coast and occupy microhabitats with distinct biotic characteristics (a coastal wetland setting for ssp. *viridulum* (Behshahr) and a wind-exposed river gorge for ssp. *vulgare* (Chalus). These differences have prompted the hypothesis that divergent selection pressures (fungal pathogen load in the Behshahr lowland versus arthropod herbivore pressure in the Chalus gorge) may have favored the evolution of phenolic antimicrobials in one population and volatile ester-based deterrents in the other (Negin and Jander, 2023). This adaptive trade-off hypothesis is consistent with the chemical data but remains speculative in the absence of field ecological surveys of pathogen and herbivore incidence, common-garden experiments, or genetic analyses. Future studies should directly test this hypothesis through: (i) reciprocal transplant or common-garden experiments to dissociate genetic from environmental contributions to chemotype expression; (ii) insecticidal and repellent bioassays of ssp. *vulgare* EO against relevant arthropod herbivores to assess whether the ester-ketone profile confers the predicted ecological function; and (iii) population-level sampling across the Caspian littoral to determine whether the thymol/linalyl acetate dichotomy is consistent or continuous.

Table 1. Essential oil composition of *Origanum vulgare* ssp. *viridulum* and ssp. *vulgare* from two geographically distinct Iranian populations.

Compound	RI*	Behshahr	Chalus
(E)-2-Hexenal	848	1.7	1.4
α-Thujene	931	1.5	1.3
α-Pinene	941	4.5	3.4
Camphene	958	0.5	1.9
1-Octen-3-ol	976	0.9	1.9
Sabinene	980	**5**	3.2
3-Octanone	984	**9.2**	**10.9**
β-Pinene	986	**11.3**	**8.4**
Myrcene	992	1.5	1.3
α-Terpinene	1022	0.5	4.7
1,8-Cineole	1038	2	2.8
(E)- β-Ocimene	1041	2.8	1.6
γ-Terpinene	1057	**13**	**16.5**
Linalool	1096	1.7	nd
1-Octen-3-yl acetate	1100	3.2	2.6
Nerol	1223	0.6	nd
Linalyl acetate	1249	Nd	**27.2**
Thymol	1284	**29.9**	nd
Carvacrol	1293	**5.2**	**6.4**
Geranyl acetate	1373	Trace	nd
trans-Caryophyllene	1431	Trace	1.4
(E)-β-Farnesene	1450	Nd	1
α-Humulene	1465	0.4	nd
(E,E)-α-Farnesene	1501	1.1	nd
Bicyclogermacrene	1509	Nd	1.1
β-Sesquiphellandrene	1533	Trace	nd
α-Cadinene	1536	1.4	nd
(E)-nerolidol	1567	0.5	nd
Caryophyllene oxide	1597	0.5	nd
Monoterpene hydrocarbons		40.6	42.3
Oxygenated monoterpenes		39.4	36.4
Sesquiterpene hydrocarbons		2.9	3.5
Oxygenated sesquiterpenes		1	0
Other compounds		15	16.8
Total (%)		98.9	99
Essential oil content (v/w %)		0.40	0.10

* RI: Retention Index, calculated relative to a homologous series of *n*-alkanes. Major compounds (≥5%) were highlighted in bold. Trace (T); not detected (nd)

Antimicrobial assessment

Analysis of variance revealed that EO antimicrobial efficacy was significantly influenced by all three experimental factors and their interactions (Table 2). The subspecies main effect exhibited the strongest influence ($F = 2360.56$, $p < 0.01$), indicating substantial differences in overall antimicrobial potency between ssp. *viridulum* and ssp. *vulgare*. Concentration ($F = 658.68$, $p < 0.01$) and pathogen identity ($F = 203.73$, $p < 0.01$) also demonstrated highly significant main effects. Critically, all two-way interactions were significant: subspecies × concentration ($F = 68.54$, $p < 0.01$), subspecies × pathogen ($F = 312.35$, $p < 0.01$), and concentration × pathogen ($F = 54.99$, $p < 0.01$), with the subspecies × pathogen interaction being particularly pronounced. The significant three-way interaction (subspecies × concentration × pathogen, $F = 43.04$, $p < 0.01$) demonstrated that dose-response relationships varied by both subspecies and pathogen type, confirming complex, context-dependent antimicrobial activity. This variance partitioning establishes that antimicrobial efficacy cannot be attributed to any single factor but rather emerges from the integrated effects of subspecies (chemical composition), bioactive compound concentration, and target pathogen biology.

Table 2. Analysis of variance of the effect of essential oils from two subspecies of *Origanum vulgare* on 10 fungal and bacterial strains at different concentrations.

SOV	DF	MS	F-Value
Subspecies	1	17729.03	2360.56**
Concentration	2	4947.04	658.68**
Pathogen	9	1530.98	203.73**
Subspecies × Concentration	2	514.74	68.54**
Subspecies × Pathogen	9	2345.89	312.35**
Concentration × Pathogen	18	412.97	54.99**
Error	120	7.51	

** Significant difference at the 1% level;Source of Variation (SOV); Degrees of Freedom (DF); Mean Square (MS)

Analysis of main effects

Reflecting the strong subspecies main effect identified in Table 2 ($F = 2360.56$, $p < 0.01$), EOs from ssp. *viridulum* and ssp. *vulgare* exhibited markedly different antimicrobial efficacies. EOs from both subspecies were evaluated for growth inhibition against a panel of bacterial and fungal

phytopathogens. *Ssp. viridulum* (Behshahr) exhibited markedly higher mean inhibition (50.07%) compared to *ssp. vulgare* (Chalus) (30.22%), representing a 65% difference in antimicrobial efficacy (Table 3).

The marked difference in antimicrobial efficacy between subspecies (50.07% for ssp. *viridulum* vs. 30.22% for ssp. *vulgare*; Table 3) directly reflects their divergent chemical profiles characterized earlier (Table 1). Ssp. *viridulum* exhibited a phenolic-dominant composition (thymol 29.9%, carvacrol 5.2%; total 35.1%), whereas ssp. *vulgare* was characterized by an ester-ketone profile (linalyl acetate 27.2%, 3-octanone 10.9%; total 38.1%). The superior activity of ssp. *viridulum* (~1.66-fold higher than ssp. *vulgare*) aligns mechanistically with its thymol-rich chemistry: thymol disrupts microbial membranes through hydrophobic interactions with lipid bilayers (Farhadi *et al*., 2024; Gan *et al*., 2025) and interferes with key metabolic enzymes including ATPases and oxidoreductases (Li *et al*., 2022; Kauser *et al*., 2024), while carvacrol enhances these effects through synergistic membrane destabilization (Dong *et al*., 2024; Gan *et al*., 2025). The 50.07% mean inhibition reflects the cumulative effect of thymol as the principal bioactive component, supported by carvacrol and minor oxygenated sesquiterpenes (1.0%, including caryophyllen oxide and (E)-nerolidol), forming a multi-component phenolic matrix optimized for antimicrobial defense (Gan *et al*., 2025).

Conversely, ssp. *vulgare*'s lower efficacy (30.22%) corresponds to its metabolic shift toward ester-ketone biosynthesis at the expense of phenolic production. Linalyl acetate, while highly volatile and effective as an insect repellent with documented neurotoxic effects on arthropods, exhibits substantially weaker direct antimicrobial potency compared to phenolic monoterpenes. Similarly, 3-octanone contributes primarily insecticidal rather than bactericidal or fungicidal activity (Alqahtani *et al*., 2023; Ferrati *et al*., 2023; Krengel *et al*., 2023). The complete absence of oxygenated sesquiterpenes in ssp. *vulgare* (0% vs. 1.0% in ssp. *viridulum*) may further limit multi-target antimicrobial function. Collectively, these compositional features suggest that ssp. *vulgare* reallocates its secondary metabolism away from antimicrobial defense toward herbivore deterrence, consistent with its volatile, ester-rich chemical ecology.

Table 3. Mean growth inhibition effect of essential oils from two subspecies of *Origanum vulgare* on several species of phytopathogens at different concentrations.

Taxa	Mean of growth inhibition (%)	Dominant compounds
O. vulgare ssp. *viridulum*	50.07[a]	Thymol, γ-terpinene, β-pinene
O. vulgare ssp. *vulgare*	30.22[b]	Linalyl acetate, γ-terpinene, 3-Octanone

Note: Superscript letters indicated that all means were significantly different from one another (Duncan's multiple range test, $p < 0.05$).

Consistent with the strong concentration effect revealed in Table 2 ($F = 658.68$, $p < 0.01$), EO antimicrobial efficacy increased progressively with increasing concentration. Antimicrobial efficacy of the combined EOs demonstrated a clear dose-dependent response (Table 4). At the lowest concentration (2 µl per Petri dish), mean pathogen growth inhibition was 30.98%. Increasing the concentrate on to 5 µl per Petri dish elevated inhibition to 40.30%, representing a ~30% increase in antimicrobial efficacy. The highest concentration tested (8 µl per Petri dish) yielded mean growth inhibition of 49.14%, an additional ~22% increase from the 5 µl dose.

All concentrations produced significantly different inhibition levels ($p < 0.05$), confirming statistically robust dose-response relationships. However, the non-linear pattern, with greater efficacy gains at lower concentration increments, reflects classical antimicrobial saturation kinetics, where initial bioactive compound availability produces steep efficacy gains while higher concentrations yield diminishing returns as pathogenic populations approach maximum susceptibility (Valero and Giner, 2006; Magi *et al*., 2015).

Table 4. Mean growth inhibition effect of three concentrations of essential oils from two subspecies of *Origanum vulgare* on several species of phytopathogens.

Essential oil concentration (µl per Petri Dish)	Mean of pathogen growth inhibition (%)
8	49.14[a]
5	40.30[b]
2	30.98[c]

Note: Superscript letters indicated that all means were significantly different from one another (Duncan's multiple range test, $p < 0.05$). The three EO volumes tested (2, 5, and 8 µl per Petri dish) were applied to plates containing approximately 20 ml of solidified agar (9 cm diameter). Assuming an EO density of approximately 0.94 g/ml, these

doses correspond approximately to 0.094, 0.235, and 0.376 mg/ml relative to agar volume, or roughly 0.01%, 0.025%, and 0.04% v/v. These values were indicative given the volatility and non-uniform diffusion of EO constituents in agar-based systems and should not be equated with solution-phase MIC values.

The highly significant pathogen main effect (Table 2) was further examined by evaluating species-specific growth inhibition across the panel of agriculturally important fungal and bacterial pathogens (Table 5). The combined EOs demonstrated marked differential efficacy, with growth inhibition ranging from 90.24% to 12.81%. A striking pattern emerged: fungal pathogens exhibited substantially higher susceptibility than bacterial pathogens. Among fungal pathogens, *S. sclerotiorum*, a devastating broad-host-range necrotrophic pathogen causing white mold in over 400 plant species including soybean, sunflower, and canola (de Castro Pereira *et al*., 2025), showed the highest inhibition at 90.24%. *M. salvinii* (72.83%), the causal agent of stem rot disease in rice, and *F. verticillioides* (69.63%), a major maize pathogen producing fumonisin mycotoxins, also demonstrated high susceptibility. *R. solani* (52.28%), responsible for damping-off and root rot across diverse crops, and *A. flavus* (37.47%), a critical food contaminant producing aflatoxins in stored grains and nuts, showed moderate to high inhibition. The mean inhibition for fungal pathogens was 64.49%. All fungal pathogens exhibited significantly different inhibition levels from one another ($p < 0.05$), indicating species-specific responses across the fungal taxa tested. The exceptional susceptibility of *S. sclerotiorum* (90.24%) relative to other fungi is particularly noteworthy given this pathogen's agricultural significance. *Sclerotinia* white mold causes severe yield losses in economically critical crops including soybean, sunflower, canola, and dry beans, with limited effective fungicidal options currently available (de Castro Pereira *et al*., 2025).

In contrast, bacterial phytopathogens exhibited markedly lower susceptibility to the EOs, with mean inhibition values substantially reduced compared to fungal strains. *X. citri*, a major pathogen causing citrus canker with significant economic impact on citrus production, showed the highest antibacterial efficacy at 23.52% mean growth inhibition. *P. carotovorum* (16.25%), a soft rot pathogen affecting multiple vegetable crops including carrots, potatoes, and onions, demonstrated the second-highest susceptibility. The remaining three bacterial strains, *A. tumefaciens* (13.46%), responsible for crown gall disease in numerous woody and herbaceous plants, *E. amylovora* (12.91%), the causative agent of fire blight in pome fruits, and *P. syringae* (12.81%), a widespread leaf spot and canker pathogen affecting diverse crops, exhibited mean growth inhibition rates clustered in the lower range.

The EOs demonstrated strong antimicrobial selectivity, with substantially greater efficacy against fungal pathogens (64.49% mean inhibition) than against bacterial pathogens (15.8% mean inhibition), a ~4.1-fold difference (Table 5). This differential susceptibility reflects fundamental differences in microbial cell architecture and compound bioavailability. Fungal cell membranes, composed primarily of ergosterol-rich lipid bilayers with comparatively simpler cell wall structures than bacteria, are highly susceptible to phenolic compound penetration and disruption. As thymol and carvacrol accumulate at fungal membrane interfaces, they progressively disrupt membrane integrity, dissipate electrochemical gradients, and inhibit membrane-bound metabolic enzymes, leading to cell death. The oxygenated sesquiterpenes in ssp. *viridulum* likely contribute synergistically through additional mechanisms including interference with ergosterol biosynthesis and enhancement of membrane fluidity disruption (Wińska *et al*., 2019; Andrade-Ochoa *et al*., 2021).

Among fungal pathogens, *S. sclerotiorum* exhibited exceptional susceptibility (90.24% mean inhibition), representing near-complete growth suppression. This extraordinary vulnerability could be linked to the pathogen's necrotrophic lifestyle, which involves high metabolic demands and extensive membrane remodeling for host colonization. These processes, along with the required secretion of lytic enzymes, are potential targets for the EO's phenolic compounds, leading to significant disruption and subsequent high inhibition (Ma *et al*., 2015). The moderate resistance of *A. flavus* may reflect evolved tolerance mechanisms including a robust melanin-enriched cell wall, active efflux pump systems (ABC transporters) capable of expelling phenolic compounds, and metabolic detoxification pathways (Tian *et al*., 2022; Satterlee *et al*., 2023).

In stark contrast, all five gram-negative bacterial pathogens showed uniformly low

inhibition despite representing diverse genera and disease syndromes. This consistent pattern highlights that the gram-negative outer membrane barrier is the primary determinant of resistance. Gram-negative bacteria possess a lipopolysaccharide (LPS)-rich outer membrane that functions as a permeability barrier, actively excluding hydrophobic compounds including thymol and carvacrol through steric hindrance and charge repulsion, thereby preventing bioavailability to the cytoplasmic membrane and intracellular targets (Maher and Hassan, 2023; Saxena *et al*., 2023). The minimal inhibition observed (15.75% mean) likely represents effects from the more volatile and lipophilic compounds (linalyl acetate and 3-octanone) that may penetrate through porins or alternative routes, or from minor phenolic components reaching sufficient concentrations to partially permeabilize the outer membrane at high doses. The uniformly low inhibition of all five bacterial pathogens should be interpreted with the important caveat that the panel comprised exclusively gram-negative species. The literature consistently demonstrates that thymol and carvacrol exhibit substantially greater activity against gram-positive bacteria, for which the outer membrane barrier is absent. The present findings therefore characterize the antibacterial profile of these EOs specifically within the gram-negative phytopathogen context and should not be generalized to antibacterial activity broadly.

Table 5. Mean growth inhibition of 10 species of phytopathogens by the essential oils of two subspecies of *Origanum vulgare.*

	Pathogen	Mean of growth inhibition (%)
Fungal strains	*Sclerotinia sclerotiorum*	90.24[a]
	Magnaporthe salvinii	72.83[b]
	Fusarium verticillioides	69.63[c]
	Rhizoctonia solani	52.28[d]
	Aspergillus flavus	37.47[e]
Bacterial strains	*Xanthomonas citri*	23.52[f]
	Pectobacterium carotovorum	16.25[g]
	Agrobacterium tumefaciens	13.46[h]
	Erwinia amylovora	12.91[h]
	Pseudomonas syringae	12.81[h]

Note: Means followed by the same superscript letter were not significantly different (Duncan's multiple range test, $p < 0.05$).

Analysis of interactions

According to Table 2, both the concentrations of the EO and the specific subspecies of *O. vulgare* significantly influenced the mean percentage of pathogen growth inhibition. Furthermore, the strong subspecies × concentration interaction indicates that the dose-response relationship of the EO is highly dependent on the subspecies from which it was extracted (Table 6).

The EO of ssp. *viridulum* consistently demonstrated superior antimicrobial efficacy across all tested concentrations compared to that of ssp. *vulgare*. At the highest concentration (8 µl per plate), ssp. *viridulum* achieved the maximum inhibition rate (62.24%), whereas ssp. *vulgare* exhibited a markedly lower inhibition of 37.04% at the same concentration. This trend persisted at intermediate (5 µl) and lowest (2 µl) doses, where ssp. *viridulum* showed 49.64% and 38.32% inhibition, respectively, compared to 30.97% and 23.65% for ssp. *vulgare*. Duncan's multiple range test confirmed that all mean values differed significantly ($p \leq 0.05$), indicating that both concentration and subspecies exerted distinct and independent influences on antimicrobial performance.

Table 6. Comparison of the mean interactive effect of essential oil concentration and plant subspecies on the mean percentage of pathogen growth inhibition.

Treatment (concentration × subspecies)	Mean growth inhibition (%)
8 µl × ssp. *viridulum*	62.24[a]
5 µl × ssp. *viridulum*	49.64[b]
2 µl × ssp. *viridulum*	38.32[c]
8 µl × ssp. *vulgare*	37.04[d]
5 µl × ssp. *vulgare*	30.97[e]
2 µl × ssp. *vulgare*	23.65[f]

Note: Means followed by the same superscript letter were not significantly different (Duncan's multiple range test, $p < 0.05$).

The comparative analysis of the interactive effects of subspecies × pathogen type revealed clear differential patterns of susceptibility among the tested microorganisms (Table 7). Overall, both EOs exhibited strong inhibitory potential against fungal species, while bacterial strains displayed markedly lower sensitivity. Among the fungi, *S. sclerotiorum* emerged as the most susceptible pathogen, showing complete growth inhibition (100%) when exposed to the oil of ssp. *vulgare* and 80.49% inhibition with ssp. *viridulum*. *M. salvinii* and *F. verticillioides* also responded strongly, with *M. salvinii* inhibited by 97.40% under ssp. *viridulum* treatment and 48.23% under ssp. *vulgare*, whereas *F. verticillioides* showed 82.97% inhibition with ssp. *viridulum* and 56.30% with ssp. *vulgare*. Similarly, *R. solani* displayed high susceptibility to ssp. *viridulum* (76.43%) but

substantially lower sensitivity to ssp. *vulgare* (38.13%). The antifungal effect was comparatively moderate against *A. flavus*, which exhibited 57.04% inhibition with ssp. *viridulum* and 17.90% with ssp. *vulgare*, indicating its relative tolerance among the tested fungi.

In contrast, bacterial pathogens demonstrated a distinct pattern characterized by consistently lower inhibition percentages. The most affected bacterial species was *X. citri*, inhibited by 38.13% under ssp. *viridulum* and only 8.90% under ssp. *vulgare*. *P. carotovorum* followed a similar trend, with inhibition of 35.43% for ssp. *viridulum* and 7.57% for ssp. *vulgare*. Moderate suppression was observed against *A. tumefaciens* (17.37% for ssp. *viridulum*; 9.62% for ssp. *vulgare*), while *E. amylovora* (11.72 and 14.10%) and *P. syringae* (11.87 and 13.76%) were the least responsive, indicating high intrinsic tolerance.

Table 7. Comparison of the mean interactive effect of plant subspecies and pathogen type on the mean percentage of pathogen growth inhibition.

Treatment (subspecies × pathogen)	Mean growth inhibition (%)
ssp. *vulgare* × *Sclerotinia sclerotiorum*	100.00^{a}
ssp. *viridulum* × *Magnaporthe salvini*	97.40^{b}
ssp. *viridulum* × *Fusarium verticillioides*	82.97^{c}
ssp. *viridulum* × *Sclerotinia sclerotiorum*	80.49^{c}
ssp. *viridulum* × *Rhizoctonia solani*	76.43^{d}
ssp. *viridulum* × *Aspergillus flavus*	57.04^{e}
ssp. *vulgare* × *Fusarium verticillioides*	56.30^{e}
ssp. *vulgare* × *Magnaporthe salvini*	48.23^{f}
ssp. *viridulum* × *Xanthomonas citri*	38.13^{g}
ssp. *vulgare* × *Rhizoctonia solani*	38.13^{gh}
ssp. *viridulum* × *Pectobacterium carotovora*	35.43^{i}
ssp. *vulgare* × *Aspergillus flavus*	17.90^{j}
ssp. *viridulum* × *Agrobacterium tumefaciens*	17.37^{j}
ssp. *vulgare* × *Erwinia amylovora*	14.10^{k}
ssp. *viridulum* × *Pseudomonas syringae*	13.76^{k}
ssp. *vulgare* × *Pseudomonas syringae*	11.87^{kl}
ssp. *viridulum* × *Erwinia amylovora*	11.72^{kl}
ssp. *vulgare* × *Agrobacterium tumefaciens*	9.62^{lm}
ssp. *vulgare* × *Xanthomonas citri*	8.90^{m}
ssp. *vulgare* × *Pectobacterium carotovora*	7.57^{m}

Note: Means followed by the same superscript letter were not significantly different (Duncan's multiple range test, $p < 0.05$).

The significant concentration × pathogen interaction identified in Table 2 indicates that dose-response patterns varied substantially among pathogen species. Analysis of pathogen-specific responses across the three EO concentrations revealed distinct patterns between fungal and bacterial pathogens (Table 8). Fungal pathogens exhibited substantial concentration-dependent increases in growth inhibition, with efficacy gains ranging from moderate to dramatic as concentration increased from 2 to 8 µl per Petri dish. In contrast, bacterial pathogens showed minimal dose-response effects, with inhibition remaining low across all concentrations tested.

Among fungal pathogens, *S. sclerotiorum* demonstrated the most pronounced response, achieving 71% inhibition at 2 µl and reaching complete growth suppression (100% inhibition) at both 5 and 8 µl concentrations. This saturation at 5 µl indicated that moderate EO concentrations are sufficient for total control of this economically devastating white mold pathogen. *M. salvinii* and *F. verticillioides* exhibited similar dose-response trajectories, with inhibition increasing from 65% and 61% at 2 µl to 81% at 8 µl for both species, respectively. These parallel responses suggest comparable susceptibility mechanisms among these agriculturally important fungal pathogens. *R. solani* showed a steeper dose-response gradient, with inhibition increasing from 32% at 2 µl to 57% at 5 µl and 69% at 8 µl, indicating that this pathogen requires higher EO concentrations to achieve substantial control. Most notably, *A. flavus* demonstrated the steepest concentration-dependent response among fungi, with inhibition escalating from 21% at 2 µl to 27% at 5 µl and reaching 65% at 8 µl (~3-fold increase). This dramatic response at the highest concentration suggests that *Aspergillus*, while more resistant than other fungi at low concentrations, becomes increasingly vulnerable as active compound availability surpasses a critical threshold concentration.

Bacterial pathogens, conversely, exhibited minimal concentration-dependent responses. *X. citri* showed the highest bacterial inhibition across all concentrations (19%, 24%, 27% at 2, 5, and 8 µl, respectively), yet the absolute gains remained modest (8 percentage points total increase). *P. carotovorum* demonstrated essentially flat dose-response (15%, 18%, 17%), with no meaningful efficacy improvement at higher concentrations. Similarly, *E. amylovora*, *P. syringae*, and *A. tumefaciens* all showed minimal responses, with inhibition ranging from 8–9% at 2 µl to only 17–18% at 8 µl. This uniformly poor dose-response across bacterial species, despite fourfold increases in EO concentration, indicates that the fundamental barrier to antibacterial efficacy (the gram-negative outer membrane) is not overcome by simply increasing phenolic compound availability.

Table. 8. Mean interactive effect of concentration on the percentage of growth inhibition for studied pathogens.

Pathogen	Mean % growth inhibition		
	Concentration 2 µl	Concentration 5 µl	Concentration 8 µl
Sclerotinia sclerotiorum	71	100	100
Fusarium verticillioides	61	70	81
Magnaporthe salvinii	65	72	81
Rhizoctonia solani	32	57	69
Aspergillus flavus	21	27	65
Xanthomonas citri	19	24	27
Pectobacterium carotovorum	15	18	17
Erwinia amylovora	8	15	18
Pseudomonas syringae	8	13	17
Agrobacterium tumefaciens	9	15	17

Several methodological limitations of this study warrant explicit acknowledgment. The use of agar disc diffusion for antifungal activity and agar well diffusion for antibacterial activity, while widely applied in phytochemistry and plant pathology research (Magaldi *et al*., 2004; Valgas *et al*., 2007), carries inherent constraints for hydrophobic EO constituents. The diffusion of lipophilic EO constituents through aqueous agar is constrained by compound-specific partition coefficients and molecular size; in disc diffusion, volatilization from the impregnated disc adds a vapour-phase delivery component absent in well diffusion. The two assay formats therefore operate through partially distinct physical mechanisms, and direct quantitative comparison of inhibition values between the fungal and bacterial datasets should be made cautiously. The bacterial panel comprised exclusively gram-negative species; given the well-established greater susceptibility of gram-positive bacteria to thymol and carvacrol, the antibacterial findings cannot be generalized beyond the gram-negative phytopathogen context. Furthermore, no standard fungicide or bactericide positive controls were included, precluding benchmarking of EO efficacy against established management standards within this experimental system. Standardized broth microdilution assays for MIC determination, conducted according to CLSI or EUCAST protocols, with subsequent MBC/MFC assessment, would enable concentration-defined activity thresholds and are recommended as a priority for follow-up work, particularly for the most active subspecies–pathogen combinations identified here.

This comparative study elucidates the antimicrobial potential of *O. vulgare* EOs from two Iranian subspecies, highlighting their distinct chemotypes and biocontrol applications. GC-MS analysis identified ssp. *viridulum* (Behshahr) as a thymol-dominant chemotype (29.9% thymol, 5.2% carvacrol, 0.40% v/w yield) and ssp. *vulgare* (Chalus) as a linalyl acetate-dominant chemotype (27.2% linalyl acetate, 10.9% 3-octanone, 0.10% v/w yield). These chemical profiles drove differential efficacy, with ssp. *viridulum* achieving superior mean inhibition (50.07%) compared to ssp. *vulgare* (30.22%). Notably, both EOs exhibited strong fungal selectivity (64.49% mean inhibition), with *S. sclerotiorum* showing exceptional susceptibility (90.24%, 100% at 5 µl), contrasting with minimal bacterial inhibition (15.75%) due to gram-negative lipopolysaccharide barriers. The thymol-rich ssp. *viridulum* EO emerges as a potent natural antifungal for post-harvest control, seed treatment, and greenhouse applications, addressing 20–40% global crop losses amid synthetic pesticide resistance concerns. However, ineffective bacterial control necessitates integrated strategies, such as membrane permeabilizers or nanoemulsions. This study's novelty lies in linking Iranian chemotypes to efficacy against key phytopathogens, guiding EO standardization and germplasm selection for sustainable agriculture. Future research should explore in planta trials, formulation optimization, and genetic underpinnings of chemotypic variation to enhance practical deployment.

Conflict of Interest

The authors do not have any potential conflict of interest to declare.

References

Adams, RP (2017). Identification of essential oil components by gas chromatography/mass spectrometry (ed. 4.1). *Allured Publ Crop Carol Steam, IL*.

Agliassa, C and Maffei, ME (2018). *Origanum vulgare* terpenoids induce oxidative stress and reduce the feeding activity of Spodoptera littoralis. Int. J. Mol. Sci., 19: 2805.

Alqahtani, A; Abdelhameed, MF; Abdou, R; Ibrahim, AM; Dawoud, M; Alasmari, SM; El Raey, MA and

Attia, HG (2023). Mechanistic action of linalyl acetate: Acyclic monoterpene isolated from bitter orange leaf as anti-inflammatory, analgesic, antipyretic agent: Role of TNF-α, IL1β, PGE2, and COX-2. Indust. Crops Prod., 203: 117131.

Andi, SA and Maskani, F (2021). Essential oil chemical diversity of twenty Iranian *Origanum vulgare* L. subsp. *viride* populations. Biochem. Syst. Ecol., 98: 104323.

Andrade-Ochoa, S; Chacón-Vargas, KF; Sánchez-Torres, LE; Rivera-Chavira, BE; Nogueda-Torres, B and Nevárez-Moorillón, GV (2021). Differential antimicrobial effect of essential oils and their main components: Insights based on the cell membrane and external structure. Membranes, 11: 405.

Aslam, MN; Khaliq, H; Zhao, H; Moosa, A; Maqsood, A; Farooqi, MA; Bilal, MS; Mahmood, T and Mukhtar, T (2025). Thymol as a novel plant-derived antibacterial agent for suppressing xanthomonas citri pv. malvacearum in cotton. Curr. Microbiol., 82: 99.

Ayaz, M; Li, CH; Ali, Q; Zhao, W; Chi, YK; Shafiq, M; Ali, F; Yu, XY; Yu, Q and Zhao, JT (2023). Bacterial and fungal biocontrol agents for plant disease protection: Journey from lab to field, current status, challenges, and global perspectives. Molecules, 28: 6735.

Azimzadeh, Z; Hassani, A; Mandoulakani, BA; Sepehr, E and Morshedloo, MR (2023). Intraspecific divergence in essential oil content, composition and genes expression patterns of monoterpene synthesis in *Origanum vulgare* subsp. *vulgare* and subsp. *gracile* under salinity stress. BMC Plant Biol., 23: 380.

Chouhan, S; Sharma, K and Guleria, S (2017). Antimicrobial activity of some essential oils-present status and future perspectives. Med., 4: 58.

Crocoll, C (2011). *Biosynthesis of the phenolic monoterpenes, thymol and carvacrol, by terpene synthases and cytochrome P450s in oregano and thyme* Jena, Friedrich-Schiller-Universität Jena, Diss., 2011].

Crocoll, C; Asbach, J; Novak, J; Gershenzon, J and Degenhardt, J (2010). Terpene synthases of oregano (*Origanum vulgare* L.) and their roles in the pathway and regulation of terpene biosynthesis. Plant Mol. Biol., 73: 587-603.

de Castro Pereira, R; Borges, DL; Neto, JJD; Lobo Jr, M; da Silva Torres, R and Pedrini, H (2025). White mold and *Sclerotinia sclerotiorum* detection: An ensemble deep learning approach. Smart Agric. Technol., 101459.

de Sousa, DP; Damasceno, ROS; Amorati, R; Elshabrawy, HA; de Castro, RD; Bezerra, DP; Nunes, VRV; Gomes, RC and Lima, TC (2023). Essential oils: Chemistry and pharmacological activities. Biomolecules, 13: 1144.

Dong, H; You, Y; Wang, N; Wang, M; Song, T; He, Y; Zou, Y; He, Y; Peng, T and Mei, L (2024). Development of amphipathic derivatives of thymol and carvacrol as potent broad-spectrum antibacterial agents. Eur. J. Med. Chem., 276: 116716.

Escobar, A; Perez, M; Romanelli, G and Blustein, G (2020). Thymol bioactivity: A review focusing on practical applications. Arab. J. Chem., 13: 9243-9269.

Farhadi, K; Rajabi, E; Varpaei, HA; Iranzadasl, M; Khodaparast, S and Salehi, M (2024). Thymol and carvacrol against *Klebsiella*: anti-bacterial, anti-biofilm, and synergistic activities-a systematic review. Front. Pharmacol., 15: 1487083.

Ferrati, M; Spinozzi, E; Baldassarri, C; Maggi, F; Pavela, R; Canale, A; Petrelli, R and Cappellacci, L (2023). Efficacy of *Mentha aquatica* L. essential oil (Linalool/Linalool Acetate Chemotype) against insect vectors and agricultural pests. Pharm., 16: 633.

Gan, C; Langa, E; Wang, G; Van Bambeke, F; Ballestero, D and Pino-Otín, MR (2025). Mechanisms of action and resistance prevention of synergistic thymol and carvacrol combinations with antibiotics in *Staphylococcus aureus* and *Acinetobacter baumannii*. Nat. Prod. Bioprospect., 15: 36.

Godfray, HCJ; Mason-D'Croz, D and Robinson, S (2016). Food system consequences of a fungal disease epidemic in a major crop. Philos. Trans. R. Soc. Lond. B Biol. Sci., 371: 20150467.

Gruľová, D; Caputo, L; Elshafie, HS; Baranová, B; De Martino, L; Sedlák, V; Gogaľová, Z; Poráčová, J; Camele, I and De Feo, V (2020). Thymol chemotype *Origanum vulgare* L. essential oil as a potential selective bio-based herbicide on monocot plant species. Molecules, 25: 595.

Hao, Y; Guo, X; Yang, R; Yan, Y; Sun, M; Li, H; Bai, H; Cui, H; Li, J and Shi, L (2022). Unraveling the biosynthesis of carvacrol in different tissues of *Origanum vulgare*. Int. J. Mol. Sci., 23: 13231.

Hou, J; Zhang, J; Zhang, B; Jin, X; Zhang, H and Jin, Z (2020). Transcriptional analysis of metabolic pathways and regulatory mechanisms of essential oil biosynthesis in the leaves of *Cinnamomum camphora* (L.) Presl. Front. Gen., 11: 598714.

Kauser, S; Raj, N; Ahmedi, S and Manzoor, N (2024). Mechanistic insight into the membrane disrupting properties of thymol in Candida species. Microbe, 2: 100045.

Kesraoui, S; Andrés, MF; Berrocal-Lobo, M; Soudani, S and Gonzalez-Coloma, A (2022). Direct and indirect effects of essential oils for sustainable crop protection. Plants, 11: 2144.

Krause, ST; Liao, P; Crocoll, C; Boachon, B; Förster, C; Leidecker, F; Wiese, N; Zhao, D; Wood, JC and Buell, CR (2021). The biosynthesis of thymol, carvacrol, and thymohydroquinone in Lamiaceae proceeds via cytochrome P450s and a short-chain dehydrogenase. Proc. Natl. Acad. Sci., 118: e2110092118.

Krengel, F; Pavela, R; Ocampo-Bautista, F and Guevara-Fefer, P (2023). Acaricidal and insecticidal activity of essential oils obtained from the aerial

parts of three Mexican Bursera species. Environ. Sci. Pollut. Res., 30: 122717-122725.

Lange, BM; Wildung, MR; Stauber, EJ; Sanchez, C; Pouchnik, D and Croteau, R (2000). Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. Proc. Natl. Acad. Sci., 97: 2934-2939.

Larkov, O; Zaks, A; Bar, E; Lewinsohn, E; Dudai, N; Mayer, AM and Ravid, U (2008). Enantioselective monoterpene alcohol acetylation in *Origanum*, *Mentha* and *Salvia* species. Phytochem., 69: 2565-2571.

Leyva-López, N; Gutiérrez-Grijalva, EP; Vazquez-Olivo, G and Heredia, JB (2017). Essential oils of oregano: Biological activity beyond their antimicrobial properties. Molecules, 22: 989.

Li, Q; Huang, KX; Pan, S; Su, C; Bi, J and Lu, X (2022). Thymol disrupts cell homeostasis and inhibits the growth of *Staphylococcus aureus*. Contrast Media Mol. Imaging, 2022: 8743096.

Ma, B; Ban, X; Huang, B; He, J; Tian, J; Zeng, H; Chen, Y and Wang, Y (2015). Interference and mechanism of dill seed essential oil and contribution of carvone and limonene in preventing sclerotinia rot of rapeseed. Plos One, 10: e0131733.

Magaldi, S; Mata-Essayag, S; De Capriles, CH; Pérez, C; Colella, M; Olaizola, C and Ontiveros, Y (2004). Well diffusion for antifungal susceptibility testing. Int. J. Infect. Dis., 8: 39-45.

Magi, G; Marini, E and Facinelli, B (2015). Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A *Streptococci*. Front. Microbiol., 6: 165.

Maher, C and Hassan, KA (2023). The gram-negative permeability barrier: tipping the balance of the in and the out. MBio, 14: e01205-01223.

Negin, B and Jander, G (2023). Convergent and divergent evolution of plant chemical defenses. Curr. Opin. Plant Biol., 73: 102368.

Paterson, G (1982). British Pharmacopoeia 1980. Can. Med. Assoc. J., 126: 514.

Rehman, R;, Hanif, MA; Mushtaq, Z and Al-Sadi, AM (2016). Biosynthesis of essential oils in aromatic plants: A review. Food Rev. Int., 32: 117-160.

Satterlee, T; McDonough, CM; Gold, SE; Chen, C; Glenn, AE and Pokoo-Aikins, A (2023). Synergistic effects of essential oils and organic acids against *Aspergillus flavus* contamination in poultry feed. Toxins, 15: 635.

Saxena, D; Maitra, R; Bormon, R; Czekanska, M; Meiers, J; Titz, A; Verma, S and Chopra, S (2023). Tackling the outer membrane: facilitating compound entry into Gram-negative bacterial pathogens. Npj Antimicrob. Resist., 1: 17.

Tian, F; Woo, SY; Lee, SY; Park, SB; Zheng, Y and Chun, HS (2022). Antifungal activity of essential oil and plant-derived natural compounds against *Aspergillus flavus*. Antibiotics, 11: 1727.

Tian, L; Wang, X; Liu, R; Zhang, D; Wang, X; Sun, R; Guo, W; Yang, S; Li, H and Gong, G (2021). Antibacterial mechanism of thymol against *Enterobacter sakazakii*. Food Control, 123: 107716.

Valcárcel, F; Olmeda, AS; González, MG; Andrés, MF; Navarro-Rocha, J and González-Coloma, A (2021). Acaricidal and insect antifeedant effects of essential oils from selected aromatic plants and their main components. Front. Agr., 3: 662802.

Valero, M and Giner, M (2006). Effects of antimicrobial components of essential oils on growth of *Bacillus cereus* INRA L2104 in and the sensory qualities of carrot broth. Int. J. Food Microbiol., 106: 90-94.

Valgas, C; Souza, SMD; Smânia, EF and Smânia Jr, A (2007). Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol., 38: 369-380.

Wang, N; Sundin, GW; Fuente, LDL; Cubero, J; Tatineni, S; Brewer, MT; Zeng, Q; Bock, CH; Cunniffe, NJ and Wang, C (2024). Key challenges in plant pathology in the next decade. Phytopathol., 114: 837-842.

Wińska, K; Mączka, W; Łyczko, J; Grabarczyk, M; Czubaszek, A and Szumny, A (2019). Essential oils as antimicrobial agents-myth or real alternative? Molecules, 24: 2130.

Xiao, Z; Li, Q; Niu, Y; Zhou, X; Liu, J; Xu, Y and Xu, Z (2017). Odor-active compounds of different lavender essential oils and their correlation with sensory attributes. Ind. Crops Prod., 108: 748-755.

Yap, P; Yiap, B; Ping, H and Lim, S (2014). Essential oils, a new horizon in combating bacterial antibiotic resistance. Open Microbiol. J., 8: 6–14.

Yavasoglu, SI; Wood, MJ; Alkhaibari, AM; Touray, M and Butt, T (2023). Potential of 3-octanone as a lure and kill agent for control of the Brown garden snail. J. Invertebr. Pathol., 198: 107920.

Zaks, A; Davidovich-Rikanati, R; Bar, E; Inbar, M and Lewinsohn, E (2008). Biosynthesis of linalyl acetate and other terpenes in lemon mint (*Mentha aquatica* var. citrata, Lamiaceae) glandular trichomes. Isr. J. Plant Sci., 56: 233-244.

Zamuner, CFC; Marin, VR; Dilarri, G; Hypolito, GB; Sass, DC and Ferreira, H (2023). Oregano essential oil and its main components thymol and carvacrol as alternatives to control citrus canker. Front. Agr., 5: 1148969.