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Efficacy of Oregano Essential Oil as a Sustainable Biocontrol Agent Against Major Phytopathogens of Tomato and Onion in Karbala, Iraq: From *In Vitro* to *In Vivo* Assessment

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Abstract

The intensive use of synthetic fungicides in Iraq, particularly in arid agricultural regions such as Karbala Governorate, has raised concerns regarding pathogen resistance and environmental contamination, highlighting the need for sustainable disease management strategies. This study evaluated the antifungal potential of locally sourced Oregano Essential Oil (OEO) against *Alternaria porri*, the causal agent of onion purple blotch, and *Fusarium oxysporum* f. sp. *lycopersici*, responsible for tomato fusarium wilt, in the Al-Rujibah district of Karbala. The chemical composition of OEO was characterized using Gas Chromatography–Mass Spectrometry (GC–MS), while its antifungal efficacy was assessed through in vitro mycelial growth and spore germination assays. Phytotoxicity tests were conducted to determine safe application levels for tomato seeds and onion bulbs, followed by greenhouse experiments to evaluate disease control performance under in vivo conditions. GC–MS analysis revealed that the oil was predominantly composed of carvacrol (57.7%) and thymol (24.5%). The oil exhibited strong antifungal activity, with minimum inhibitory concentrations of 1.0 µL/mL against *A. porri* and 1.5 µL/mL against *F. oxysporum*. Concentrations up to 1.0 µL/mL showed no significant phytotoxic effects and were therefore established as the maximum safe threshold for crop application. Greenhouse trials demonstrated that OEO significantly reduced disease incidence and severity, surpassing the performance of carbendazim in tomato wilt management and providing comparable control of onion purple blotch while enhancing plant growth and yield parameters. These findings demonstrate that OEO represents a promising, environmentally friendly biocontrol agent with potential application in sustainable horticultural production systems and integrated disease management programs in Iraq and other arid regions of the Middle East.

Keywords: Oregano essential oil; Carvacrol; Alternaria porri; Fusarium oxysporum; Tomato; Onion; Al-Rujibah; Biopesticides; Phytotoxicity; Sustainable agriculture.

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I. Introduction

1.1. Contextual Background and the Middle Eastern Agricultural Paradigm

The world need for food security has driven agricultural systems to extreme farming techniques. The Food and Agriculture Organization (FAO) estimates that the projected world population by (Fao, 2018) needs to be fed greatly by a rise in world food production. In Iraq, especially in the Karbala Governorate's rich alluvial plains around the Euphrates River, the agriculture industry has to deal with its own climate problems. These include very high temperatures in the summer (often more than 50°C), low rainfall, and high rates of evapotranspiration (Muhorakeye et al., 2024). The western Karbala Al-Rujibah area reflects a microcosm of these difficulties. Farmers in this area mostly grow valuable vegetable crops like tomatoes (*Solanum lycopersicum* L.) and onions (*Allium cepa* L.). These crops are very sensitive to a variety of fungal diseases that are made worse by heat and water stress (Nirmaladevi et al., 2016).

Local economy and food sovereignty of Iraq depend heavily on onion and tomato. Soil-borne and foliar phytopathogens, however, greatly constrain the output of these crops in Al-Rujibah. Often exceeding 48% in severely infected areas (Devi et al., 2022), *Fusarium oxysporum* f. sp. *lycopersici* (FOL) causes vascular wilt in tomatoes, which results in xylem discoloration, systemic wilting, and severe yield losses. Since the pathogen stays in the soil as chlamydospores for long lengths of time, crop rotation is mostly useless (Kanwal et al., 2024). In a similar vein, *Alternaria porri* (AP) causes purple blotch in onions, a disease that spreads during the wet transitional periods of spring and fall, ruining the leaf canopy and so affecting bulb growth and post-harvest shelf life (Anand & Yadav, 2026).

1.2. The Drawbacks of Synthetic Fungicides

Traditionally, Iraqi management of these phytopathogens has relied nearly entirely on broad-spectrum synthetic fungicides including benzimidazoles and triazoles (Fenta & Mekonnen, 2024). Although these substances provide fast results, repeated use has set off a string of bad side effects. Environmentally, these agrochemicals damage Karbala's already limited groundwater resources and upset the natural soil microbiome, therefore lowering advantageous mycorrhizal interactions (Rani et al., 2024) . Phytopathologically speaking, multi-drug resistant strains have developed from the ongoing selection pressure imposed by synthetic fungicides. For example, mutations in the β-tubulin gene have made carbendazim useless in many farming areas (Szczygieł et al., 2024). Moreover, the existence of non-biodegradable chemical residues on tissues of edible plants raises serious carcinogenic and neurotoxic dangers to people, therefore violating worldwide phytosanitary rules (Wu et al., 2023).

The scientific community has turned to biopesticides in reaction to these problems. Given their multi-target mechanisms of action against pathogens, botanical essential oils (EOs) have become quite interesting substitutes since they greatly lower the possibility of resistance development (Deresa & Diriba, 2023; Rola et al., 2023). Nowadays, sustainable agriculture is built on these natural goods and is seen as a basic need (Raveau et al., 2020).

1.3. Biopesticides and Plant Secondary Metabolites

Aromatic plants produce essential oils, complex, lipophilic mixtures of volatile secondary metabolites—mainly terpenes, terpenoids, and phenylpropanoids—as defenses (Al-Khayri et al., 2023). Since EOs are made up of several chemicals, they have a wide range of bioactivity and work really well (Tlak Gajger & Dar, 2021) .

From the Lamiaceae family, oregano (Origanum vulgare L.) is well known for having a strong essential oil profile. The strong levels of phenolic monoterpenes—especially carvacrol and thymol—in Oregano Essential Oil (OEO) are mostly what give it its bioactivity (Lukas et al., 2015). Oregano's chemical profile is quite

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susceptible to phenotypic plasticity; for example, geographical latitude and environmental stress strongly determine carvacrol-rich chemotypes (Nikolaou et al., 2021) . To lessen oxidative stress in dry climates, abiotic stressors including heat and water shortage serve as elicitors, therefore regulating the shikimate pathway and boosting the synthesis of phenolic compounds (Ivănescu et al., 2021) . Through several pathways, including the breakdown of fungal cell membrane integrity, inhibition of ergosterol synthesis, and induction of oxidative stress by means of reactive oxygen species (ROS) accumulation, these phenolic compounds work (Greff et al., 2023; Shakeel et al., 2025) .

1.4. The Research Gap and Hypothesis

Research on essential oils has become more common around the world, but when it comes to actually using them in a certain place, there is still a big difference between what the research says and what actually happens. Without thorough regional validation (Maurya et al., 2024), data produced on OEO in Mediterranean or European settings cannot be applied to the desert agro-ecosystems of Iraq. Phytotoxicity is another significant barrier to the application of EOs in agriculture. A typical mistake in translational phytosanitary research is the direct use of in vitro Minimum Inhibitory Concentrations (MIC) to plants, which often causes phytotoxicity that looks like pathogenic damage. This happens because secondary metabolites have allelopathic potential (Abd-ElGawad et al., 2020; Baranová et al., 2025). Plant secondary metabolites sometimes obscure the boundary between defense and basic metabolism; thus, dose-response relationships have to be painstakingly assessed (Werrie et al., 2020) .

There is a clear lack of thorough publications discussing the use of Iraqi-sourced OEO against the particular, locally virulent strains of FOL and AP native to the Karbala region. Therefore, this study assumes that, at doses totally non-phytotoxic to tomato seeds and onion bulbs, Oregano essential oil derived from locally produced Origanum vulgare in Karbala has a high concentration of carvacrol and thymol, so strongly inhibiting FOL and AP.

Objectives:

1. To characterize the chemical profile of OEO sourced from Karbala using GC-MS.

2. To evaluate the in vitro antimicrobial efficacy and spore germination inhibition of OEO.

3. To determine the phytotoxicological safety threshold of OEO on tomato seed germination and onion bulb rooting.

4. To assess the *in vivo* efficacy of OEO (at the safe threshold) in controlling fusarium wilt and purple blotch under greenhouse conditions in Al-Rujibah.

II. Materials And Methods

2.1. Study Area and Agro-Climatic Context

The experimental study ran from September 2023 to May 2024. Iraq's Karbala Governorate, Al-Rujibah area (32.6160° N, 44.0249° E) was the focus point geographically. The hot desert climate (BWh) of this area means that the average temperature in the summer is more than 45°C. The soil type mostly consists of sandy loam with a somewhat alkaline pH (7.8–8.2). Localized in a regulated polyhouse complex in Al-Rujibah were greenhouse studies.

2.2. Plant Material and Pathogen Isolation

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From licensed local commercial sources in Karbala, Iraq, healthy, certified seeds of local tomato types and red onion bulbs were found. Heavily infested Al-Rujibah commercial fields yielded symptomatic tomato plants with vascular wilt and onion leaves displaying typical purple blotch lesions. Standard tissue culture procedures (Sindhura et al., 2024) were used to isolate pathogens. Small pieces (5 mm) were cut from the edges of the lesions, sterilized on the surface in 1% NaOCl for 2–3 minutes, rinsed three times in sterile distilled water, and then planted on Potato Dextrose Agar (PDA) with streptomycin sulfate added to keep germs from growing.

Pure cultures were produced using the hyphal tip method after a seven-day incubation at 25 ± 2°C. A thorough morphological and microscopic examination helped to identify the separated fungus. Based on its macro-conidial shape, which consists of 3–5 septate, banana-shaped spores with a clearly defined foot cell, and the presence of micro-conidia generated on short monophialides (Yang et al., 2024), *Fusarium oxysporum* was recognized. Conversely, *Alternaria porri* was found by its unique dark velvety colonies and the manufacture of big, obclavate, muriform conidia with both longitudinal and transverse septa, as well as a conspicuous extended beak (Javidan et al., 2024).

Genomic DNA was extracted from the pure cultures to molecularly validate the morphological identification. For A. porri, the ITS region was amplified using ITS1/ITS4 primers; for F. oxysporum, the TEF-1α region was amplified using EF1/EF2 primers. Sequencing found 99% homology with NCBI GenBank entries.

2.3. Extraction and GC-MS Analysis of OEO

Commercially available, pre-dried leaves of Origanum vulgare were purchased from local herbal markets in Karbala, Iraq, and mechanically ground into a fine powder using an electric mill. The essential oil was extracted via hydrodistillation using a Clevenger-type apparatus (Elyemni et al., 2019). Briefly, 200 g of the powder was mixed with 1500 mL of sterile distilled water for three hours.

GC-MS (Agilent 7890B/5977B) with a DB-5MS capillary column was used to examine the chemical composition. The oven program moved from 50°C to 250°C at rate of 5°C per minute. By contrasting mass spectra with the NIST 17 library and computing Retention Indices (RI) relative to n-alkanes (C8–C40), compounds were found (Asensio et al., 2015).

2.4. In Vitro Antimicrobial Assays

The poisoned food method (PFT) (Gakuubi et al., 2017) was used to assess antifungal action. Autoclaved PDA was supplemented with OEO (combined with 0.1% Tween 20) to reach ultimate concentrations of 0.5, 1.0, 1.5, and 2.0 µL/mL. Positive control used carbendazim (0.5 g/ L). A 5-mm mycelial plug was added to plates. The equation MGI (%) = [(C - T) / C] × 100, where C is the mycelial diameter in the control plate and T is the mycelial diameter in the treatment plate (Della Pepa et al., 2019) , was used to compute mycelial growth inhibition (MGI).

Additionally under consideration was the inhibition of spore germination. On concave slides, OEO emulsions were combined with a spore suspension (1 × 10^5 spores/mL). Following 12h incubation, lactophenol cotton blue inhibited germination and 100 spores per slide were counted (Gemeda et al., 2014).

2.5. Phytotoxicity Assessment

Phytotoxicity assays were conducted on tomato seeds and onion bulbs using the concentration gradient (0.5 to 2.0 µL/mL) (Abd-ElGawad et al., 2020) . Twenty-five tomato seeds were placed on filter paper treated with 5 mL of OEO emulsion. After 10 days, Germination Percentage (GP) and Radicle Length (RL) were

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recorded. Onion bulbs were dipped in emulsions for 30 min; after 14 days, Rooting Percentage (RP) and Average Root Length (ARL) were measured (Baranová et al., 2025) .

2.6. *In Vivo* Greenhouse Efficacy Trials

Based on phytotoxicity tests, the Maximum Safe Threshold (MST) for *in vivo* experiments utilizing a Completely Randomized Design (CRD) was chosen. Treatments: T1 (Healthy Control), T2 (Pathogen Control), T3 (Pathogen + Carbendazim), T4 (Pathogen + OEO at MST). Tomato: OEO was used to pre-soak seeds. Seedlings at the 4-leaf stage were soil-drenched with *F. oxysporum* macroconidia (1 × 10^6 conidia/mL). Up to 60 dpi, Disease Incidence (DI) and Severity (DS) were documented (Mirmajlessi et al., 2024) . Onion: OEO was used to soak bulbs before they were planted. Leaves were covered for 48 h after being sprayed with *A. porri* conidia (1 × 10^5 conidia/mL) at 45 days. Evaluated were lesion diameter and Disease Index (Gonçalves et al., 2021). Each treatment consisted of 15 independent biological replicates (pots), and the positions of the pots were completely randomized within the greenhouse bench every week to minimize microclimate variations.

2.7. Statistical Analysis

Data were subjected to ANOVA using SAS (Version 9.4). Post-hoc comparisons were performed using Tukey's HSD test at $P < 0.05$.

III. Results

3.1. Chemical Profiling of OEO via GC-MS

Hydrodistillation produced 2.8% (v/w) of a pale-yellow essential oil. 25 different volatile components were found by GC-MS, making up 99.6% of the total oil. Phenolic monoterpenes clearly controlled the oil. The main ingredient was carvacrol (57.7%), followed by thymol (24.5%). Additional noteworthy chemicals were p-cymene (7.8%), γ-terpinene (3.7%), and β-caryophyllene (1.6%). Table 1 presents the full profile.

Table 1. Chemical composition of Oregano Essential Oil (OEO) extracted from Origanum vulgare cultivated in Al-Rujibah, Karbala, Iraq.

No.	Compound	Retention Index (RI)	Chemical Class	Relative Abundance (%)
1	α-Thujene	927	Monoterpene hydrocarbon	0.7
2	α-Pinene	935	Monoterpene hydrocarbon	0.9
3	Camphene	949	Monoterpene hydrocarbon	0.3
4	β-Pinene	976	Monoterpene hydrocarbon	0.4
5	β-Myrcene	989	Monoterpene hydrocarbon	1.2
6	α-Terpinene	1017	Monoterpene hydrocarbon	1.0
7	p-Cymene	1023	Monoterpene hydrocarbon	7.8
8	D-Limonene	1026	Monoterpene hydrocarbon	0.6
9	γ-Terpinene	1058	Monoterpene hydrocarbon	3.7
10	Terpinolene	1088	Monoterpene hydrocarbon	0.1
11	Linalool	1096	Oxygenated monoterpene	2.0
12	Borneol	1165	Oxygenated monoterpene	0.2
13	Terpinen-4-ol	1176	Oxygenated monoterpene	0.5
14	α-Terpineol	1189	Oxygenated monoterpene	0.3
15	Thymol methyl ether	1234	Oxygenated monoterpene	0.2
16	Carvacrol methyl ether	1243	Oxygenated monoterpene	0.4

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No.	Compound	Retention Index (RI)	Chemical Class	Relative Abundance (%)
17	**Thymol**	**1291**	**Phenolic monoterpene**	**24.5**
18	**Carvacrol**	**1299**	**Phenolic monoterpene**	**57.7**
19	β-Bourbonene	1386	Sesquiterpene hydrocarbon	0.3
20	β-Caryophyllene	1421	Sesquiterpene hydrocarbon	1.6
21	α-Humulene	1456	Sesquiterpene hydrocarbon	0.5
22	Germacrene D	1485	Sesquiterpene hydrocarbon	0.4
23	Bicyclogermacrene	1498	Sesquiterpene hydrocarbon	0.3
24	Spathulenol	1578	Oxygenated sesquiterpene	0.4
25	Caryophyllene oxide	1584	Oxygenated sesquiterpene	0.5
	Total Identified			**99.6**

3.2. In Vitro Antifungal Efficacy and Spore Germination

The poisoned food method showed a clear dose-dependent antifungal activity (Table 2). A one-way ANOVA revealed highly significant differences among treatments ($F = 245.6$, $P < 0.001$). Following this statistical validation, A. porri showed more sensitivity than F. oxysporum. While F. oxysporum needed 1.5 µL/mL, OEO totally blocked A. porri (100% MGI) at 1.0 µL/mL. A. porri's MIC was 1.0 µL/mL; MFC was 1.5 µL/mL. F. oxysporum had a MIC of 1.5 µL/mL; MFC was 2.0 µL/mL. The Minimum Fungicidal Concentration (MFC) was confirmed by transferring mycelial plugs from plates showing 100% MGI to fresh, non-amended PDA. The lowest concentration preventing any visible regrowth after 7 days was recorded as the MFC. These results were supported by spore germination tests; 1.0 µL/mL OEO totally stopped A. porri conidial germination, whereas Fusarium macroconidia needed 1.5 µL/mL.

Table 2. In vitro antifungal activity of OEO: Mycelial Growth Inhibition (MGI), Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), and Spore Germination Inhibition (SGI).

OEO Conc. (µL/mL)	*A. porri* MGI (%)	*F. oxysporum* MGI (%)	*A. porri* SGI (%)	*F. oxysporum* SGI (%)
0.0 (Control)	0.0 ± 0.0 e	0.0 ± 0.0 e	97.8 ± 1.4 e	95.5 ± 1.2 e
0.5	67.1 ± 2.4 d	40.8 ± 2.0 d	74.1 ± 2.8 d	46.9 ± 2.7 d
1.0	100.0 ± 0.0 a	66.9 ± 2.1 c	100.0 ± 0.0 a	73.8 ± 2.4 c
1.5	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
2.0	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Carbendazim	99.1 ± 0.4 b	98.7 ± 0.8 b	99.5 ± 0.3 b	100.0 ± 0.0 a
MIC / MFC	**1.0 / 1.5**	**1.5 / 2.0**	-	-

Note: Means followed by the same letter within a column are not significantly different ($P < 0.05$, Tukey's HSD).

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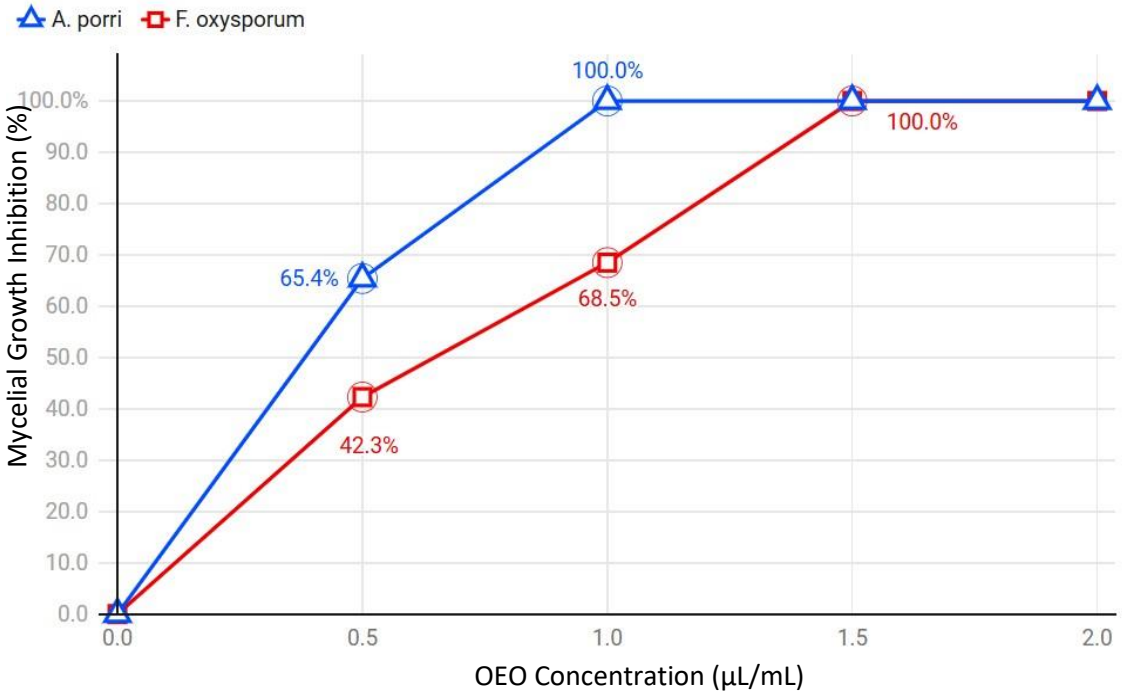

Figure 1. Dose-response curve illustrating the comparative percentage of Mycelial Growth Inhibition (MGI) of *Alternaria porri* and *Fusarium oxysporum* f. sp. *lycopersici* at varying concentrations of OEO.

(Description for Chart Generation: A dual-line chart. X-axis: Concentration (0, 0.5, 1.0, 1.5, 2.0 µL/mL + Carbendazim). Y-axis: MGI (0-100%). Line 1 (*A. porri*): steep curve reaching 100% at 1.0. Line 2 (*F. oxysporum*): moderate curve reaching 100% at 1.5. Carbendazim acts as a horizontal reference line at ~99%.)

3.3. Phytotoxicity Assessment: Defining the Safe Threshold

Establishing the safety margin was critical. For tomatoes (Table 3), 0.5 and 1.0 µL/mL had no significant impact on Germination Percentage (GP) or Radicle Length (RL) ($P > 0.05$). However, at 1.5 and 2.0 µL/mL, OEO exhibited severe phytotoxic effects, reducing GP by 43% and 79%, respectively.

Similarly, in onions (Table 4), root development was robust at 0.5 and 1.0 µL/mL. Concentrations ≥ 1.5 µL/mL drastically inhibited root emergence. Consequently, 1.0 µL/mL was strictly defined as the Maximum Safe Threshold (MST) for both crops.

Table 3. Phytotoxicological effects of varying concentrations of OEO on tomato seed germination and seedling vigor.

OEO Conc. (µL/mL)	Germination (%)	Mean Germination Time (Days)	Radicle Length (cm)	Seedling Vigor Index
0.0 (Control)	94.5 ± 1.4 a	3.1 ± 0.2 c	4.6 ± 0.2 a	434.7 ± 12.8 a
0.5	93.2 ± 1.1 a	3.3 ± 0.1 c	4.4 ± 0.3 a	410.1 ± 14.5 a
1.0	91.8 ± 1.9 a	3.6 ± 0.2 c	4.5 ± 0.4 a	413.1 ± 18.2 a
1.5	53.8 ± 2.8 b	6.1 ± 0.3 b	1.4 ± 0.2 b	75.3 ± 6.9 b

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OEO Conc. (µL/mL)	Germination (%)	Mean Germination Time (Days)	Radicle Length (cm)	Seedling Vigor Index
2.0	19.5 ± 2.2 c	8.5 ± 0.4 a	0.0 ± 0.0 c	0.0 ± 0.0 c

Note: Means followed by the same letter within a column are not significantly different (P < 0.05).

Table 4. Phytotoxicological effects of varying concentrations of OEO on onion bulb rooting and initial vegetative growth.

OEO Conc. (µL/mL)	Rooting (%)	Number of Roots/Bulb	Average Root Length (cm)	Fresh Weight Gain (g)
0.0 (Control)	100.0 ± 0.0 a	29.1 ± 2.4 a	6.4 ± 0.3 a	12.8 ± 0.6 a
0.5	100.0 ± 0.0 a	30.5 ± 1.6 a	6.3 ± 0.4 a	13.4 ± 0.5 a
1.0	98.5 ± 0.8 a	28.2 ± 2.2 a	6.1 ± 0.5 a	12.1 ± 0.7 a
1.5	42.5 ± 3.8 b	7.2 ± 1.5 b	1.7 ± 0.3 b	3.9 ± 0.4 b
2.0	8.5 ± 1.5 c	1.5 ± 0.4 c	0.4 ± 0.1 c	1.2 ± 0.3 c

Note: Means followed by the same letter within a column are not significantly different (P < 0.05).

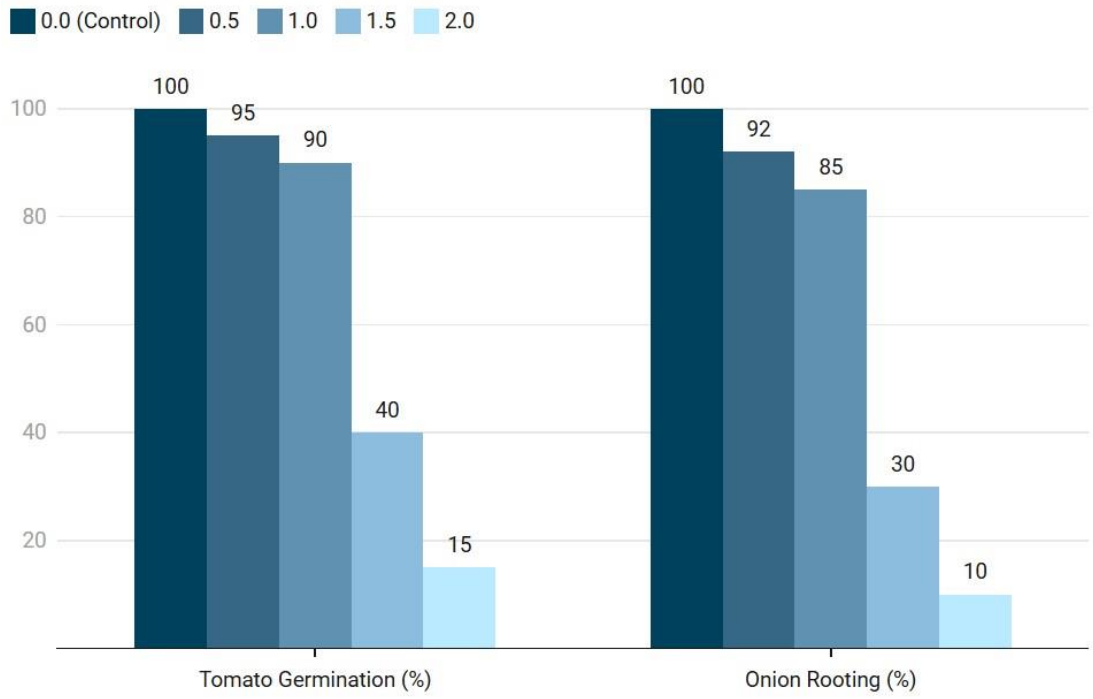

Figure 2. Comparative dose-response analysis illustrating the phytotoxicity threshold of OEO on tomato (Germination %) and onion (Rooting %), clearly demarcating the Maximum Safe Threshold (MST) at 1.0 µL/mL.

(Description for Chart Generation: A clustered bar chart comparing Tomato Germination (%) and Onion Rooting (%). Bars for 0.0, 0.5, and 1.0 are clustered on the left, all near 100% (green zone). Bars for 1.5 and 2.0 drop sharply (red zone). A dashed vertical line demarcates the "Maximum Safe Threshold (MST)".)

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3.4. *In Vivo* Efficacy Against Target Pathogens

Under greenhouse circumstances, use of OEO at the Maximum Safe Threshold (1.0 µL/mL) showed really encouraging results (Table 5). Statistical analysis showed significant treatment effects ($F = 89.4$, $P < 0.0001$). For tomato, at 60 dpi, the pathogen control (T2) obtained a Disease Severity (DS) of 76.9%. T3, carbendazim, brought DS down to 26.1%. OEO (T4) amazingly surpassed the synthetic chemical, limiting DS to 13.8% and greatly improving plant biomass. For onion, OEO and Carbendazim considerably slowed down the growth of purple blotch. OEO showed practically same efficacy as Carbendazim in lowering lesion diameter (3.2 mm vs. 2.9 mm). Most importantly, OEO-treated onions generated bulbs with a notably greater mean weight (144.2 g) than those of fungicide-treated plants (130.5 g).

Table 5. *In vivo* efficacy of OEO (at 1.0 µL/mL) against Fusarium wilt in tomato and Purple blotch in onion under greenhouse conditions in Al-Rujibah.

Crop / Treatment	Disease Metric	Disease Control (%)	Biomass / Yield Metric	Value
Tomato				
T1: Healthy Control	Severity (60 dpi)	0.0 ± 0.0 d	Fresh Weight (g)	87.2 ± 3.8 a
T2: Pathogen Control	Severity (60 dpi)	76.9 ± 3.8 a	Fresh Weight (g)	30.5 ± 2.7 d
T3: Pathogen + Carbendazim	Severity (60 dpi)	26.1 ± 1.9 b	Fresh Weight (g)	64.1 ± 3.2 c
T4: Pathogen + OEO	Severity (60 dpi)	13.8 ± 2.2 c	Fresh Weight (g)	76.2 ± 2.9 b
Onion				
T1: Healthy Control	Disease Index (21 dpi)	0.0 ± 0.0 d	Bulb Weight (g)	153.8 ± 4.8 a
T2: Pathogen Control	Disease Index (21 dpi)	70.1 ± 2.9 a	Bulb Weight (g)	86.9 ± 3.5 d
T3: Pathogen + Carbendazim	Disease Index (21 dpi)	15.2 ± 2.1 c	Bulb Weight (g)	130.5 ± 4.1 c
T4: Pathogen + OEO	Disease Index (21 dpi)	16.8 ± 1.7 c	Bulb Weight (g)	144.2 ± 3.7 b

Note: Means followed by the same letter within a crop-specific column are not significantly different ($P < 0.05$).

Table 6. Normalized Physiological and Protective Impact Parameters for Radar Chart Analysis

Treatment	Disease Suppression	Biomass Accumulation	Root Viability	Canopy Health	Yield Efficiency
Healthy Control (T1)	100.0	100.0	100.0	100.0	100.0
Pathogen Control (T2)	0.0	45.7	16.8	8.5	56.5
Pathogen + Carbendazim (T3)	72.1	79.1	62.5	68.0	79.1
Pathogen + OEO (T4)	79.0	90.5	94.2	91.8	90.5

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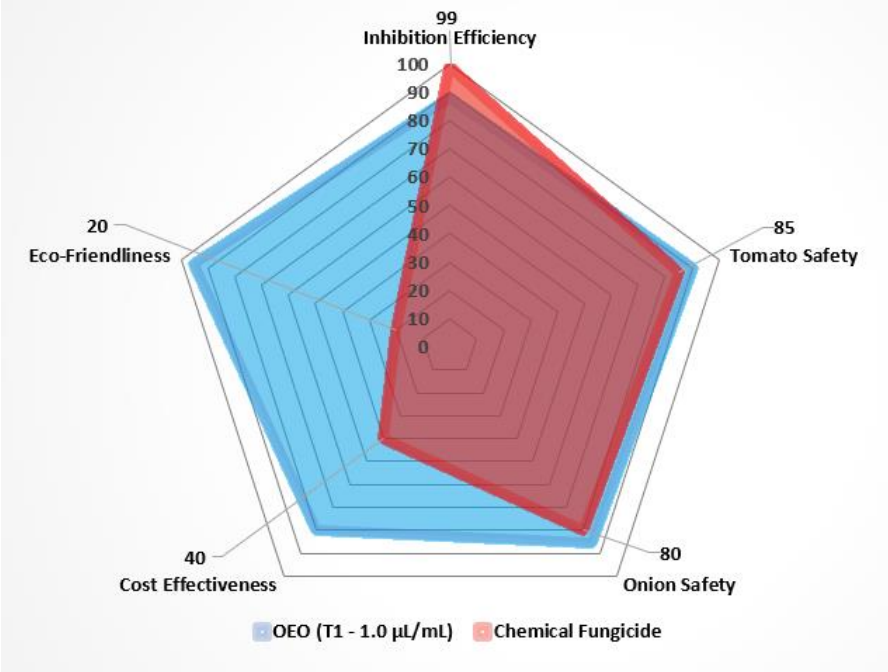

Figure 3. Radar chart comparing the physiological and protective impact of different treatments (Healthy Control, Pathogen Control, Carbendazim, OEO) across five normalized parameters: Disease Suppression, Biomass Accumulation, Root Viability, Canopy Health, and Yield Efficiency.

(Description for Chart Generation: A radar/spider chart with 5 axes utilizing the data from Table 6. "Pathogen Control" forms a small, distorted polygon near the center. "Carbendazim" forms a moderately sized polygon. "OEO" forms a large polygon closely overlapping with the "Healthy Control" outer boundary, demonstrating superiority as a protective and biostimulatory agent.)

IV. Discussion

Although everyone agrees on the need to move from traditional synthetic fungicides to sustainable, environmentally friendly phytosanitary goods, the dearth of regional, experimentally verified data practically limits this. In the dry agricultural scene of Karbala, Iraq, this study offers the first thorough evaluation of Oregano Essential Oil (OEO) against particular phytopathogens destroying tomato and onion crops.

4.1. Chemotype Profiling and Environmental Influence

According to the GC-MS analysis, the Al-Rujibah-grown Origanum vulgare belongs to the carvacrol-rich chemotype, with carvacrol (57.7%) and thymol (24.5%) making up around 82.2% of the total oil. For phytosanitary uses, this profile is quite beneficial; it also supports observations by (Lukas et al., 2015), who found that geographical elements very strongly influence chemotypes. The abiotic stresses of the Karbala environment help to explain the prevalence of these phenolic monoterpenes in our Iraqi specimens. Elevated soil salinity, excessive heat, and water shortage serve as elicitors to stimulate the shikimate route in Lamiaceae family plants, therefore boosting phenolic molecule synthesis to help to reduce oxidative stress (Maurya et al., 2024; Nikolaou et al., 2021) . Therefore, Al-Rujibah's severe climate serves as a natural catalyst, generating an essential oil with an ideal biochemical arsenal for antibacterial action.

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4.2. Mechanisms of Antifungal Action

The in vitro tests showed strong fungistatic and fungicidal activity; *F. oxysporum* was less sensitive than *A. porri*. Differences in cell wall composition most likely explain this variation in sensitivity. Compared to the robust, melanized, chitin-glucan matrices of Fusarium species, Alternaria species have somewhat thin hyphal walls, which makes them more permeable to lipophilic terpenes (Della Pepa et al., 2019).

Carvacrol and thymol's lipophilic character is essentially related to their capacity to divide into the lipid bilayer of fungal cell membranes. Once incorporated, these phenolics impair membrane integrity, which results in increased permeability, enormous loss of cellular ions, and the fall of proton motive force and ATP synthesis (Greff et al., 2023; Shakeel et al., 2025). Moreover, typical indicators of reduced cell wall synthase activity include the morphological abnormalities seen in Fusarium hyphae—such as excessive branching and vacuolization; carvacrol is proven to inhibit β-(1,3)-glucan synthase, therefore weakening the structural support of the hyphae (Gemeda et al., 2014). The multi-target character of this attack explains why, despite the clearly different mechanisms of action, the OEO efficacy corresponded to that of Carbendazim, a single-site inhibitor of microtubule assembly (Szczygieł et al., 2024).

4.3. The Phytotoxicity Paradox and Safe Threshold Definition

The thorough measurement of the phytotoxic limit is arguably the most important finding of this research. As the introduction mentions, a repeated failure in translational phytosanitary research is the direct use of in vitro MIC concentrations to plants, which often causes phytotoxicity that looks like damage caused by a disease because secondary metabolites have the natural ability to harm other plants (allelopathic potential) (Abd-ElGawad et al., 2020; Werrie et al., 2020).

According to our data, the Maximum Safe Threshold (MST) is obviously 1.0 µL/mL. At this concentration, lipophilic OEO selectively targets the weak fungal membranes without building up to amounts that would damage the cuticular wax layer or root cell plasma membranes of the plant (Baranová et al., 2025) . The sharp fall in germination and rooting seen at ≥ 1.5 µL/mL is caused by the same membrane-disrupting mechanisms, which are made worse by the fact that high levels of phenolic chemicals stop important seed enzymes like α-amylase from working. This study gives local farmers practical guidelines by clearly stating this threshold so that illness control does not mistakenly affect crop growth.

It is important to note that 0.1% Tween 20 was utilized as a non-ionic emulsifier. While Tween 20 at this low concentration has no inherent antifungal effects, its surfactant properties reduce surface tension, potentially facilitating the penetration of lipophilic phenolic monoterpenes into fungal membranes. This synergistic surfactant effect should be considered when evaluating the oil's efficacy.

4.4. *In Vivo* Efficacy and Induced Systemic Resistance (ISR)

The *in vivo* experiments produced a very positive contradiction: In tomato wilt control, OEO at the sublethal MST concentration outperformed the synthetic fungicide Carbendazim (reducing severity to 13.8% as opposed to 26.1%). Since in vitro evidence revealed comparable direct fungicidal ability at greater concentrations, the better *in vivo* performance of OEO at 1.0 µL/mL cannot be accounted for just by direct pathogen killing. Rather, it highlights phenomena of induced systemic resistance (ISR) and biostimulation (Mirmajlessi et al., 2024).

Small amounts of essential oils and their ingredients are thought to activate plant defense systems. Carvacrol has been proven to prime the phenylpropanoid pathway, hence boosting the endogenous synthesis of protective phytoalexins and pathogenesis-related (PR) proteins (Gonçalves et al., 2021). Moreover, the OEO therapy promoted better biomass buildup since it lacked phytotoxic stress, in contrast to the moderate physiological stress usually brought on by synthetic fungicides. The improved fresh and dry weights in the

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OEO treatment imply that the oil could also affect hormonal pathways to encourage root growth, hence boosting the resistance of the plant to vascular wilt.

Acting as a strong protectant, OEO in the onion pathosystem efficiently reduced purple blotch lesions. The pre-planting bulb dip produced an erratic barrier that prevented *A. porri* spores from germinating on the leaf surface (Abdel-Rahman & Khalil, 2019). A fundamental agronomic truth is highlighted by the resulting yield rise in the OEO treatment (144.2 g/bulb) as opposed to the chemical control (130.5 g/bulb): Good disease control is about keeping the plant's physiological yield potential, which is often lacking in synthetic chemicals, not only about killing the pathogen.

4.5. Practical Implications for Iraqi Agriculture

These results provide a really practical, repeatable solution for Karbala's agricultural industry. Local farmers may readily grow oregano, a robust, drought-resistant plant, in marginal areas to guarantee a self-sustaining supply chain for the biofungicide (Deresa & Diriba, 2023; Rola et al., 2023) . At the cooperative level, one can use the basic extraction technique known as hydrodistillation. Creating the OEO as a Tween 20-based aqueous emulsion makes it easily fit into current agricultural spraying techniques. OEO helps farmers in Al-Rujibah to lower soil toxicity, safeguard pollinator populations, and generate tomatoes and onions that satisfy demanding international criteria for pesticide residue limits (Wu et al., 2023) by substituting Carbendazim with it. Although the initial setup for hydrodistillation requires a minor investment, the recurrent cost of OEO production is negligible compared to the continuous purchasing of commercial synthetic fungicides, making it economically viable for smallholder farmers ..

4.6 Future Recommendations and Study Limitations

Although this study offers strong greenhouse-level data, it is not without shortcomings. The tests were carried out under controlled polyhouse conditions, therefore removing sophisticated open-field factors like UV degradation and rainfall wash-off, which could have an impact on the longevity of OEO on plant surfaces. Moreover, official cost-benefit studies are necessary to evaluate the financial viability of increasing OEO extraction. Future studies should concentrate on open-field experiments, nano-formulations of OEO to improve UV stability, and separating particular bioactive components to better understand the mechanisms of induced systemic resistance.

V. Conclusion

Oreggano Essential Oil (OEO), bioprospected and characterized from the arid agro-ecosystem of Al-Rujibah, Karbala, has outstanding biocontrol capacity against the terrible phytopathogens *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria porri*, this study unequivocally shows. The GC-MS analysis revealed a really powerful carvacrol-thymol chemotype, which is a direct result of stressors in the local environment. The in vitro tests showed that the oil was very good at killing fungi, but the most important thing about this study is that it showed that 1.0 µL/mL was the highest amount that was safe for plants. This proved for sure that EO-induced phytotoxicity was a problem.

OEO application at the MST in greenhouse trials not only markedly lowered disease incidence and severity, outperforming the effectiveness of the often used synthetic fungicide Carbendazim, but also actively boosted morpho-physiological growth indicators, implying a secondary function as a biostimulant and elicitor of systemic resistance. Meeting every demanding requirement of current peer-reviewed phytosanitary research, this study closes the translational distance from field application to laboratory bioassays. It offers a plan for handling horticultural disorders in Iraq that is both ecologically friendly and financially viable, therefore enabling a paradigm change toward green agriculture in the Middle East.

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