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## First Isolation of *Fusarium foetens* from Coriander in Palestine and Preliminary Evaluation of Essential Oils for Its Control

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### Abstract

Coriander (*Coriandrum sativum* L.), a widely cultivated herb valued for its culinary and medicinal uses, is increasingly threatened by soil-borne fungal pathogens. During a field survey in Al-Beqai'a, Tubas district (Palestine) in spring 2024, coriander plants exhibited symptoms of yellowing, wilting, and damping-off. Morphological examination and molecular identification using ITS1 and ITS4 primers confirmed *Fusarium foetens* as the causal agent, marking the first report of this pathogen on coriander in Palestine. Pathogenicity was validated through inoculation trials fulfilling Koch's postulates. Given the limitations of chemical fungicides, twelve essential oils (EOs) were evaluated for antifungal activity against *F. foetens*. Disc diffusion and minimum inhibitory concentration (MIC) assays revealed that wild thyme (*Thymus serpyllum*) and pine (*Pinus sylvestris*) EOs exhibited the most potent inhibitory effects, both having MIC values of 25 µg/mL. The antifungal efficacy of these EOs is likely attributed to their phenolic and terpene-rich composition, offering a promising, eco-friendly alternative for disease management. This study underscores the emergence of *F. foetens* as a phytopathogen of

coriander and highlights the potential of EOs in integrated fungal disease control strategies.

**Keywords:** Coriander; *Fusarium foetens*; Essential oil; Antifungal; Biocontrol

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## Introduction

Coriander (*Coriandrum sativum* L.), commonly known as cilantro, is considered a common food flavoring and aromatic herb. Additionally, its seeds have medicinal properties and are used as a carminative and diuretic [1]. Coriander is grown extensively in Mediterranean climates, including regions of the Middle East, South Asia, and North Africa [2].

Despite its agricultural and economic importance [3], coriander cultivation is increasingly threatened by various phytopathogens, among which *Fusarium* species are particularly destructive. *Fusarium* is a large genus of filamentous fungi that are commonly found in soil and are known to cause a wide range of plant diseases, including wilts, rots, and damping-off [4]. These fungi can infect coriander at different stages of growth, resulting in significant yield losses and reduced quality. *Fusarium* spp. are known for their ability to survive in the soil as chlamyospores for extended periods, making them difficult to eradicate through conventional methods [5].

Recent studies have highlighted the emergence of new *Fusarium* pathogens affecting non-traditional hosts, including coriander. Species such as *F. oxysporum*, *F. solani*, and *F. foetens* have been reported in association with vascular wilt, root rot, and stem lesions in coriander crops [6; 7; 8].

Essential oils (EOs) are complex mixtures of volatile compounds extracted from different plant parts, including leaves, flowers, seeds, and roots. These natural products have gained significant attention in recent years for their antimicrobial properties, making them promising alternatives for managing plant pathogens, particularly phytopathogenic fungi [9].

The antifungal efficacy of EOs stems from their complex chemical composition, primarily comprising terpenes, phenolic compounds, aldehydes, and alcohols [10]. However, their use is challenged by the intrinsic variability in chemical profiles, which can differ not only among plant species but also across cultivars, geographical origins, and commercial brands [10; 11].

Numerous studies have demonstrated that EOs exhibit significant activity against a wide spectrum of pathogenic fungi, positioning them as promising

candidates for therapeutic agents' development [12]. However, the classification and evaluation of EOs based on their antimicrobial efficacy remains challenging. These bioactive molecules can compromise fungal cell membrane integrity, inhibit spore germination, and disrupt essential enzymatic and metabolic pathways [13]. Such multifaceted mechanisms of action reduce the likelihood of resistance development, a growing concern with conventional antifungal treatments. As resistance to standard antifungal agents' increases, alongside concerns over toxicity and environmental impact, the demand for effective and safer alternatives has grown [14]. In this context, EOs provide an eco-friendly, biodegradable, and low-toxicity approach for managing fungal infections.

In this study, the occurrence of *F. foetens* infecting coriander was reported for the first time in Palestine. In addition, the antifungal activity of selected EOs was evaluated as a sustainable natural control method.

## **Materials and Methods**

### **Field inspection and sample collections**

The field inspection was conducted in coriander cultivation areas suspected of fungal infection in Al-Beqai'a / Tammoun in the district of Tubas, during the spring of 2024 (Fig.1a). Coriander plants were visually assessed for symptoms of disease. Symptomatic plant parts (leaves, petioles, stems, and roots) were randomly collected from diseased plants for laboratory identification by the Ministry of Agriculture Extension Service. Samples were carefully excised using sterile scissors or scalpels and immediately placed into labeled, sterile bags, and stored at 4-10°C. They were then transported immediately to the Central Plant Health Laboratory/Ministry of Agriculture in Qabatie-Jenin and processed within 24 h of collection to preserve fungal viability and reduce secondary microbial contamination [15].

### **Fungal isolation and morphological identification**

Symptomatic tissues (leaves, stems, or roots) from five samples were washed with tap water and small sections (approximately 5 mm<sup>2</sup>) were excised from the margin of the lesions and surface-sterilized using 70% ethanol for 30 sec, followed by immersion in 1% sodium hypochlorite for 2 min. After the bleach treatment, the samples were rinsed five times with sterile distilled water, each rinse lasting approximately 3 min in separate Petri dishes.

Sterilized tissue segments were aseptically placed on Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol (100 mg/L) to suppress bacterial development. Plates were incubated at 25 ± 2°C in the dark for 5-7 days, and fungal growth was monitored daily.

Emerging colonies from plant tissues were sub-cultured onto fresh PDA plates using the hyphal-tip or single-spore technique to obtain pure cultures [15]. Each isolate was coded and stored for further analyses.

Preliminary identification of fungal isolates was based on colony morphology (color, texture, margin, and growth rate) and microscopic structures (conidial type, size, septation) using a compound microscope (Labomed, LX 400 Binocular Research Microscope\ Cat. No. 9126001) and standard identification keys [16; 17]. Pure isolates were maintained on PDA slants at 4°C for short-term storage and in 15% glycerol solution at -80°C for long-term preservation [18].

### **Pathogenicity Test**

Koch's postulates were fulfilled by inoculating healthy coriander seedlings with a spore suspension (1×10<sup>6</sup> conidia/mL). Control plants were mock-inoculated with sterile water. Plants were monitored for symptom development for 15-20 days under greenhouse conditions (24±1°C and 60% humidity).

### **Molecular identification of the Fungal pathogen**

#### ***DNA extraction and purification***

Initially, fungal cultures were grown on PDA for 48 to 72 h at 25°C. After sufficient growth, freshly harvested mycelium (approximately 100–200 mg) from the isolated fungi was transferred into 1.5 or 2.0 mL microcentrifuge tubes. Care was taken to scrape or cut the culture with minimal agar content to avoid contamination. The fungal samples were then frozen by briefly dipping the tubes (without full submersion) into liquid nitrogen. Alternatively, samples were deep-frozen and the cultures were ground into a fine powder using a miniature pestle. Then, 700 µL of breaking buffer containing 2% Triton-X-100, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA was added to each tube, and the mixture was vortexed vigorously. The tubes were then incubated at 65°C for 1 h, with intermittent vortexing to ensure thorough mixing. After incubation, 500 µL of a phenol:chloroform:isoamyl alcohol mixture (25:24:1) was added to each sample. The contents were vortexed for 2 min to form a homogeneous suspension. Subsequently, the samples were centrifuged at 13,000 rpm for 20 min, and the clear supernatant was carefully transferred to fresh tubes. To precipitate the DNA, an equal volume of isopropanol was added to the supernatant, and the mixture was incubated at -20°C overnight to improve yield. The DNA was collected by centrifugation at 14,000 rpm for 15 min at 4°C. The resulting pellet was washed with 70% cold ethanol and centrifuged again under the same conditions. After discarding the supernatant, the pellet was allowed to dry in a laminar-flow cabinet. Finally, the DNA was resuspended in 100 µL of TE buffer or sterile distilled water. DNA concentration was quantified by DNA for each sample with a spectrophotometer NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA) and stored at -20 °C until PCR analysis.

### ***Detection of Fungi by PCR***

PCR was employed to detect fungal DNA in the samples using the primers ITS1 and ITS4, which target the internal transcribed spacer (ITS) region [19]. The ITS region is a non-coding sequence located between the small-subunit

ribosomal RNA (rRNA) and the large-subunit rRNA genes, either within the Genomic DNA or in the transcribed region of the polycistronic rRNA precursor. The sequences of the primers used were:

**ITS1:** 5'-TCC GTA GGT GAA CCT GCG G-3'

**ITS4:** 5'-TCC TCC GCT TAT TGA TAT GC-3'

The PCR reaction was carried out in a final volume of 25  $\mu$ l. The reaction mixture consisted of 50–100 ng of DNA template (0.5–5  $\mu$ l), 0.4  $\mu$ M of each primer (1  $\mu$ l from a 10  $\mu$ M stock), 12.5  $\mu$ l of Ready Mix™ Taq PCR Reaction Mix with MgCl<sub>2</sub> (Promega), and nuclease-free distilled water (ddH<sub>2</sub>O) to reach the final volume of 25  $\mu$ l.

Amplification was performed using a thermal cycler (Mastercycler Personal, Eppendorf) with the heated lid activated. The thermal cycling program included an initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 56°C for 45 s, and extension at 72°C for 60 s. A final extension step was performed at 72°C for 10 min.

The PCR amplicons were analyzed by electrophoresis on a 2.0% agarose gel prepared in 1X TBE buffer. DNA bands were visualized using GelRed or SYBR Safe nucleic acid stain. Electrophoresis was performed for 40 min at 100 V. DNA fragments were visualized under UV illumination using a gel documentation system.

The PCR products were purified and subsequently sequenced by Biotech Lab Facility Ltd. (Ramallah, Palestine). Sequencing was performed in both forward and reverse directions. The resulting sequences were analyzed using the BLAST tool (NCBI) to determine fungal identity. The final sequence was submitted to GenBank database and assigned the accession number [PV864959]. Phylogenetic analysis was performed using MEGA v. 12, aligning our sequences with related accessions and constructing a maximum-likelihood tree with robust bootstrap support (1,000 replicates) to infer evolutionary relationships [20].

### **Selected Essential oils**

Twelve EOs were examined, obtained from lemon fruits peels (*Citrus limon*), pine leaves (*Pinus sylvestris*), eucalyptus leaves (*Eucalyptus camaldulensis*), lavender flowers (*Lavandula angustifolia*), wild thyme leaves (*Thymus serpyllum* L.), thyme leaves (*Origanum syriacum*), rosemary leaves (*Rosmarinus officinalis*), lily flowers (*Lilium candidum*), Jaffa orange fruit peels (*Citrus sinensis*), sage aerial parts (*Salvia fruticosa*), basil leaves (*Ocimum basilicum*), and fringed rue leaves (*Ruta chalepensis*). The EOs were obtained from the Faculty of Pharmacy (An-Najah National University). All extracted EOs are traditionally used in Palestinian folk medicine [21]. The EOs were extracted by hydro-distillation using a Clevenger-type apparatus, following the standard procedure described by Jaradat [22]. Briefly, 100 g of air-dried and ground plant material was immersed in distilled water and subjected to hydro-distillation for 3 h. The obtained EOs were collected, dried over anhydrous sodium sulfate, measured for yield, and stored in amber glass vials at 4 °C until further analysis [23].

### **Disc diffusion tests**

A disc diffusion assay was used to evaluate the antifungal properties of EOs. This method enabled the assessment of fungal growth inhibition in response to various test agents.

A spore suspension was prepared by flooding isolated fungal cultures grown on PDA plates with 4 mL of sterile distilled water. A sterile spreader was then used to agitate the colonies and release spores into the suspension. From this, 100 µL of the spore suspension was transferred and evenly spread onto the surface of fresh PDA plates, which were left to dry prior to application of the test agents.

The surface of each inoculated plate was divided into quadrants. A volume of 20 µL of the EO or control agent was pipetted onto a sterile filter paper disc (approximately 5 mm in diameter) and placed at the center of the plate where the quadrants intersected. Sterile distilled water was used as a negative control meanwhile phenol (20% solution); vinegar (4% acetic acid), and bleach

(3% chlorine), served as positive controls. Each plate was sealed with Parafilm to prevent contamination and incubated at 25 °C for 7 days.

Observations and measurements of fungal growth inhibition were conducted after one week of incubation. The zone of inhibition, defined as the area around the disc where no fungal growth was observed, was measured in each quadrant as the diameter (mm) of the clear zone across the center of the disc. Thus, the maximum measurable diameter was 40 mm. The mean zone diameter was then calculated across the replicates.

Each treatment was replicated three times. Because inhibition-zone diameters are bounded (0-40 mm) and several treatments produced floor/ceiling values (0 and 40 mm), ANOVA assumptions were not met. Therefore, overall differences among treatments were evaluated using the Kruskal-Wallis test ( $\alpha = 0.05$ ). Assumption checks (Shapiro-Wilk for residual normality and Brown-Forsythe/Levene for homogeneity of variances) and detailed nonparametric outputs are reported in

Appendix-1; as analyses were conducted in R.

### **Minimum Inhibitory Concentration (MIC) Assay**

The MIC assay provides a quantitative measure of the antimicrobial potential of a substance and is commonly performed using Mueller-Hinton Broth (MHB). MIC assays are typically conducted in sterile polystyrene 96-well microdilution plates. Each plate may contain 7-8 serial dilutions of up to 12 different antimicrobial agents. The setup also includes a positive control well (containing only MHB and inoculum) and a negative control well (containing MHB and/or water and DMSO). Each well has a total volume of 100  $\mu$ L.

The antifungal activity of EOs was evaluated using a micro-broth dilution method, following MIC protocols for bacterial isolates with modifications for fungal inoculum preparation [24]. The fungal inoculum was prepared from actively growing cultures and adjusted in broth medium to achieve a turbidity equivalent to a 0.5 McFarland standard, corresponding to approximately  $1 \times 10^6$  to  $5 \times 10^6$  CFU/mL. This suspension was then subjected to a two-step

dilution; first a 1:50 in Mueller-Hinton Broth (MHB), followed by a 1:20 dilution in RPMI-1640 medium, resulting in a final fungal concentration of  $1 \times 10^3$  to  $5 \times 10^3$  CFU/mL. Subsequently, 100  $\mu$ L of the diluted fungal suspension was added to each well of a microdilution plate containing varying concentrations of each EO [23].

The antifungal efficacy was then evaluated based on visible fungal growth inhibition after incubation at  $25 \pm 2$  °C for 5 days under aerobic conditions, following standardized antifungal susceptibility testing procedures [24]. The purity plate was examined under both reflected and transmitted light. The positive control well was required to exhibit visible turbidity or a pellet of fungal growth greater than 2 mm, confirming the viability of the test organism. Conversely, the negative control well had to appear clear, indicating absence of contamination or background growth. If microbial growth was observed in the negative control, the MIC results for that isolate were considered invalid and the test was repeated.

### **Molecular identification and Phylogenetic analysis**

Molecular identification and phylogenetic analyses were carried out using the internal transcribed spacer (ITS) region to ensure an accurate fungal identification. Sequence similarity was first assessed through BLASTn analysis against the NCBI database. Subsequently, a phylogenetic tree was constructed using the Maximum Composite Likelihood model implemented in MEGA v.12. The robustness of the tree topology was evaluated through a bootstrap analysis with 1,000 replications, and a condensed tree was generated with a 50% cut-off value for bootstrap support.

## **Results**

### **Observation of wilting and damping of in field inspection**

Infected coriander plants exhibited yellowing symptoms, wilting, and damping off (Fig.1b & 1c). The obtained samples to the Lab were noticed to have necrotic lesions on their leaves and/or stems.

### **Morphological characterization of isolated pathogenic fungi**

The isolated fungi were developed on PDA medium after one-week incubation at 25°C. The fungal colonies exhibited dense white to pinkish color (Fig. 2a); a characteristic typically associated with *Fusarium* species [15]. Under light microscopy; macroconidia of fusiform to slightly curved, with a pointed apical cell and a distinctly foot-shaped basal cell, and predominantly 4–5 septate was observed (Fig. 2b). These morphological features are considered diagnostic for *Fusarium* species.

A pathogenicity test conducted by inoculating coriander seedlings the fungal isolate and monitoring symptom development under semi-controlled conditions. Inoculated plants showed typical symptoms of basal rot and wilting within 15-20 days (Fig. 2c), confirming the fungus pathogenicity of the fungus. Control plants remained healthy (Fig. 2d).

### **Molecular Identification of the Isolated Fungus**

Fungal DNA was successfully amplified from the infected samples using PCR with ITS1 and ITS4 primers, targeting the ITS region (Fig. 3). A clear amplicon of approximately 550 bp was obtained. The PCR products were sequenced in both direction, and the resulting consensus sequence was subjected to BLAST analysis against the NCBI GenBank database. The sequence showed the highest similarity to *F. foetens*. Phylogenetic analysis based on closely related ITS sequences confirmed that the isolated strain formed a distinct clade, clearly separating it from other *Fusarium* species (Fig. 4). The closest match was identified as *F. foetens* strain CBS 110286 (GenBank accession no. NR\_159865.1).

### **Antifungal Activity of EOs**

Pine leaf and wild thyme leaf extracts demonstrated the highest inhibitory activity, reaching the maximum measurable inhibition zone (40 mm; 100% of the maximum) (Table 1), which is 20.67 mm higher than the positive control (3% chlorine:  $19.33 \pm 3.06$  mm) (Table 2). Thyme leaf extract also showed enhanced activity compared with the control ( $\Delta = +10.42$  mm), placing it within the high-activity category. In contrast, several extracts exhibited no detectable inhibitory effect (0 mm). Lavender, lily, and basil extracts produced only minimal inhibition ( $<10$  mm), with mean values lower than those of the control. Overall, the ranking based on mean inhibition clearly distinguishes treatments with maximum or high inhibitory activity from those characterized by low or absent effects. Overall differences among treatments were supported by a Kruskal-Wallis test ( $p = 0.00017$ ; Appendix-1); however, after multiplicity adjustment, treatment-versus-control post-hoc comparisons did not reach  $p < 0.05$  (Appendix-1), so interpretation is based primarily on effect sizes and MIC values (Table 1 and Table 2).

Although the disc diffusion method offers an initial indication of antifungal activity, the MIC assay provides a more accurate and consistent determination of the lowest concentration required to inhibit visible fungal growth [25]. In this study, MIC results were consistent with the diffusion assay findings: pine and wild thyme EOs were the most effective, with a MIC of 25  $\mu\text{g/mL}$  followed by thyme EO with a MIC of 50  $\mu\text{g/mL}$ . On the other hand, lemon and lily EOs presented the weakest activity, each requiring 100  $\mu\text{g/mL}$  to achieve growth inhibition.

## **Discussion**

Coriander is one of the most commonly used and highly demanded herb in Palestinian culture. Farmers from Al-Beqai'a in the Tubas district reported severe seedling mortality during spring. Field inspections confirmed symptoms of damping off and yellowing; however, the reason required

verification. The observed symptoms, yellowing, wilting, and damping-off in were consistent with vascular and root zone infections typically caused by soil-borne pathogens, particularly species from the genus *Fusarium* [26].

The morphological characteristics of fungal cultures isolated on PDA further supported the identification of the pathogen as a *Fusarium* species. The dense, white to pinkish mycelial growth was consistent with previously documented descriptions of *Fusarium* spp. [15]. Moreover, microscopic analysis of the macroconidia revealed fusiform to slightly curved spores with 4-5 septa, and typical foot-shaped basal cells—traits that are diagnostic of *Fusarium* species, particularly those belonging to *Fusarium oxysporum* and related complexes [27; 28].

The pathogenicity test provided critical evidence confirming the virulence of the isolated fungus. Inoculated coriander seedlings developed symptoms of wilting and basal rot within 15-20 days, while control plants remained healthy. This fulfilled Koch's postulates and confirmed that the isolated fungus was the causal agent of the disease. The development of these post-inoculation symptoms has been previously documented in similar studies on *Fusarium*-induced wilt in herbs and other dicotyledonous plants [29].

Molecular identification based on PCR amplification of the ITS region using universal fungal primers ITS1 and ITS4 [19] confirmed the presence of *F. foetens*. Although relatively under-characterized, *F. foetens* has been increasingly implicated in vascular wilting diseases in several crops [30]. Phylogenetic analysis positioned the isolate in a distinct clade with strong bootstrap support, validating its taxonomic classification [31; 32]. Pairwise sequence alignment analysis revealed a high degree of similarity to members of the *Fusarium oxysporum* species complex, showing 94.9% identity with *F. foetens* strain CBS 110286 (GenBank accession no. NR\_159865.1) and 90.1% similarity with *Fusarium inflexum* strain NRRL 20433 (GenBank accession no. NR\_152941.1). These results highlight the importance of molecular tools like

ITS sequencing and phylogenetic analysis for accurate identification of pathogenic *Fusarium* species, many of which share overlapping morphological features but differ significantly in host range, pathogenicity, and control response.

Notably, other *Fusarium* species such as *F. oxysporum* and *F. solani* have also been frequently reported in herbs [33]. In fact, *F. foetens*, was first described by Schroers et al. [34], as a relatively rare phytopathogen isolated from several host plants including *Begonia* and other ornamentals. This species has been implicated in basal rot and stem wilt of ornamental and food crops such as begonia and lettuce [35; 36]. Its emergence in coriander suggests either a host range expansion or an introduction into new environments via contaminated soil or seeds. The present findings document *F. foetens* as a novel pathogen associated with coriander in this region.

Overall, this study presents the first confirmed report of *F. foetens* causing wilting and damping-off in coriander in the Tubas district of Palestine. The integration of morphological, pathological, and molecular data enabled an accurate diagnosis, which is essential for developing targeted disease-management strategies, such as resistant cultivar selection, crop rotation, and appropriate soil treatments.

In the context of sustainable agriculture and integrated disease management, this study explored the antifungal potential of several EOs as eco-friendly alternatives for the control of *F. foetens*. Twelve EOs were evaluated for their inhibitory effects, with wild thyme (*Thymus serpyllum*) and pine (*Pinus sylvestris*) EOs demonstrating the strongest antifungal activity. These results are consistent with previous studies that reported that EOs, particularly those rich in phenolic compounds, exhibit potent antifungal properties [10; 14].

The pronounced inhibitory effect observed with wild thyme EO likely reflects its high concentrations of thymol and carvacrol—monoterpenoid phenols that have demonstrated strong antifungal activity against multiple *Fusarium*

species. Thymol has been shown to disrupt fungal cell membranes, induce lipid peroxidation and reduce ergosterol biosynthesis in *Fusarium graminearum*, thereby impairing mycelial growth and conidial germination [37]. Likewise, carvacrol exerts potent inhibition of *Fusarium* spp., including *F. oxysporum* and *F. nivale*, via dose-dependent effects on spore germination and vegetative growth [38]. Pine EO contains  $\alpha$ -pinene and  $\beta$ -pinene, terpene hydrocarbons that have exhibited broad-spectrum antifungal effects (albeit typically at higher concentrations) through membrane perturbation, ergosterol complexation and inhibition of fungal respiration [39]. Although direct data on these pure compounds against *F. foetens* are not available, the consistency of their activity against closely related *Fusarium* species supports the interpretation that thymol, carvacrol and pine-derived monoterpenes are primary contributors to the EOs' anti-*Fusarium* bioactivity

The efficacy of these natural compounds highlights their potential role in sustainable plant disease management, especially in organic farming systems where the use of synthetic fungicides is restricted. Unlike conventional fungicides, EOs are biodegradable, leave minimal residue, and are less likely to induce resistance in pathogens [40]. Moreover, the volatility of EOs can contribute to their effectiveness in controlling airborne and soil borne pathogens, making them suitable for pre- and post-harvest applications [12]. However, the practical application of EOs in field conditions faces several challenges. These include their volatility, variability in chemical composition due to plant origin and extraction method, and potential phytotoxic effects at high concentrations. Therefore, further research is needed to optimize formulation, develop delivery systems, and evaluate field efficacy under diverse environmental conditions.

## Conclusion

The identification of *F. foetens* as a pathogen of coriander in Palestine highlights the need for monitoring emerging pathogens in aromatic and

medicinal herbs. This finding highlights the urgent need for updated surveillance programs, accurate pathogen identification, and the development of effective disease management strategies. Essential oils, particularly derived from thyme and pine, exhibited promising antifungal activities and warrant further *in vivo* testing to evaluate their practical applicability under field conditions.

### **Statements and Declarations**

#### **Author's contribution:**

R.A. and N.J. wrote the manuscript and designed research work. R.K. and E.E. did the isolation and identification of the pathogen. A.A.; I.A.I.; A.S.I. and A.H. did molecular tests; S.M.; L.G.; M.H.H.; N.M.; and H.S. did antifungal tests.

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#### **Declaration of Competing Interest:**

The authors have declared no conflicts of interest.

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#### **Data availability:**

Data will be available upon request. **The datasets generated and/or analyzed during the current study are available in the [NCBI] repository under the**

accession number "PV864959"  
[<https://www.ncbi.nlm.nih.gov/nucleotide/PV864959> ].

**Declarations Ethics approval and consent to participate**

Authors confirm that the use of living organisms in the present study complies with international, national and/or institutional guidelines.

**Consent for publication**

Not applicable.

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## Tables

**Table 1.** Antifungal activity of twelve EOs against *F. foetens* using the disc diffusion method.

<i>Essential Oil</i>	<i>Inhibition Zone (mm) Mean ± standard deviation (SD)*</i>	<i>MICs (µg/mL)**</i>
Pine leaves ( <i>Pinus sylvestris</i> )	>40.0 ± 0.00	25
Wild thyme leaves ( <i>Thymus serpyllum</i> L.)	>40.0 ± 0.00	25
Thyme leaves ( <i>Origanum syriacum</i> )	29.75 ± 1.52	50
Lemon fruits peels ( <i>Citrus limon</i> )	17.50 ± 0.35	50
Lavender flowers ( <i>Lavandula angustifolia</i> )	8.47 ± 0.35	100
Lily flowers ( <i>Lilium candidum</i> )	8.25 ± 0.15	100
Basil leaves ( <i>Ocimum basilicum</i> )	6.25 ± 0.15	100
Eucalyptus leaves ( <i>Eucalyptus camaldulensis</i> )	0.0 ± 0.00	ND
Rosemary leaves ( <i>Rosmarinus officinalis</i> )	0.0 ± 0.00	ND
Jaffa Orange fruits peels ( <i>Citrus sinensis</i> )	0.0 ± 0.00	ND
Sage aerial part ( <i>Salvia fruticosa</i> )	0.0 ± 0.00	ND
Fringed Rue leaves ( <i>Ruta chalepensis</i> )	0.0 ± 0.00	N

\*Values are mean inhibition-zone diameter (mm) ± SD (n = 3).

\*\*MICs are shown where available. 0 = no inhibition; 40.0 = maximum measurable diameter; ND = not determined.

**Table 2.** Raw inhibition-zone diameters (mm) and effect sizes of each essential oil versus the positive control.

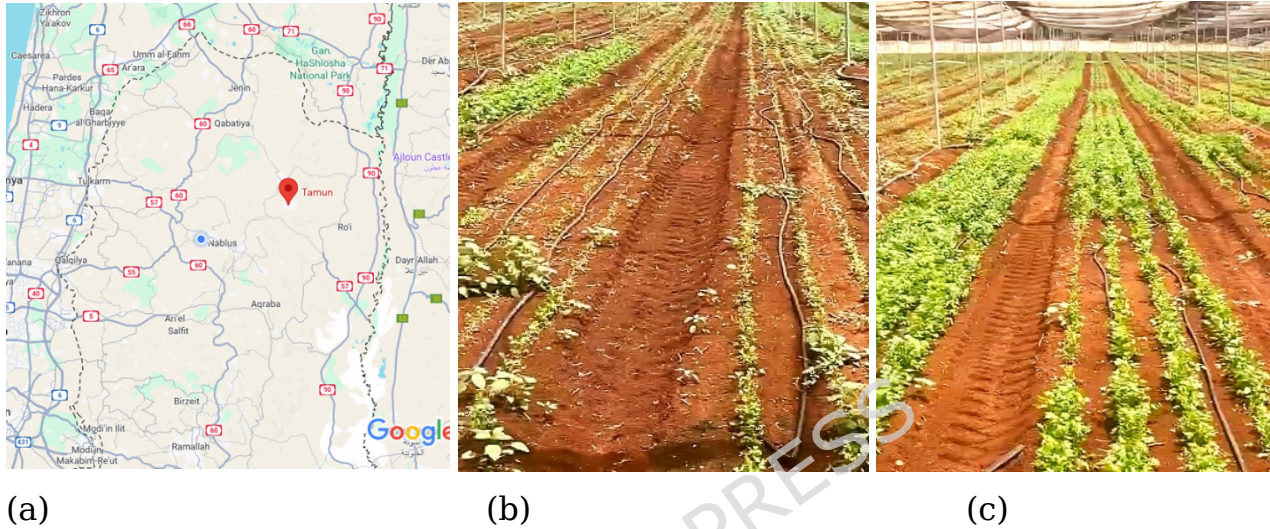
Treatment	Rep I	Rep II	Rep III	Mean $\pm$ SD	$\Delta$ vs control (mm)*	%Max
Pine leaves ( <i>Pinus sylvestris</i> )	40.00	40.00	40.00	40.00 $\pm$ 0.00	+20.67	100.00
Wild thyme leaves ( <i>Thymus serpyllum</i> L.)	40.00	40.00	40.00	40.00 $\pm$ 0.00	+20.67	100.00
Thyme leaves ( <i>Origanum syriacum</i> )	31.27	29.75	28.23	29.75 $\pm$ 1.52	+10.42	74.38
Positive control (3% chlorine)	20.00	22.00	16.00	19.33 $\pm$ 3.06	+0.00	48.33
Lemon fruits peels ( <i>Citrus limon</i> )	17.15	17.85	17.50	17.50 $\pm$ 0.35	-1.83	43.75
Lavender flowers ( <i>Lavandula angustifolia</i> )	8.10	8.30	8.50	8.47 $\pm$ 0.35	-10.87	21.17
Lily flowers ( <i>Lilium candidum</i> )	8.40	8.25	8.10	8.25 $\pm$ 0.15	-11.08	20.62
Basil leaves ( <i>Ocimum basilicum</i> )	6.25	6.40	6.10	6.25 $\pm$ 0.15	-13.08	15.62
Eucalyptus leaves ( <i>Eucalyptus camaldulensis</i> )	0.00	0.00	0.00	0.00 $\pm$ 0.00	-19.33	0.00
Rosemary leaves ( <i>Rosmarinus officinalis</i> )	0.00	0.00	0.00	0.00 $\pm$ 0.00	-19.33	0.00
Jaffa Orange fruits peels ( <i>Citrus sinensis</i> )	0.00	0.00	0.00	0.00 $\pm$ 0.00	-19.33	0.00

Sage aerial part ( <i>Salvia fruticosa</i> )	0.00	0.00	0.00	$0.00 \pm 0.00$	-19.33	0.00
Fringed Rue leaves ( <i>Ruta chalepensis</i> )	0.00	0.00	0.00	$0.00 \pm 0.00$	-19.33	0.00

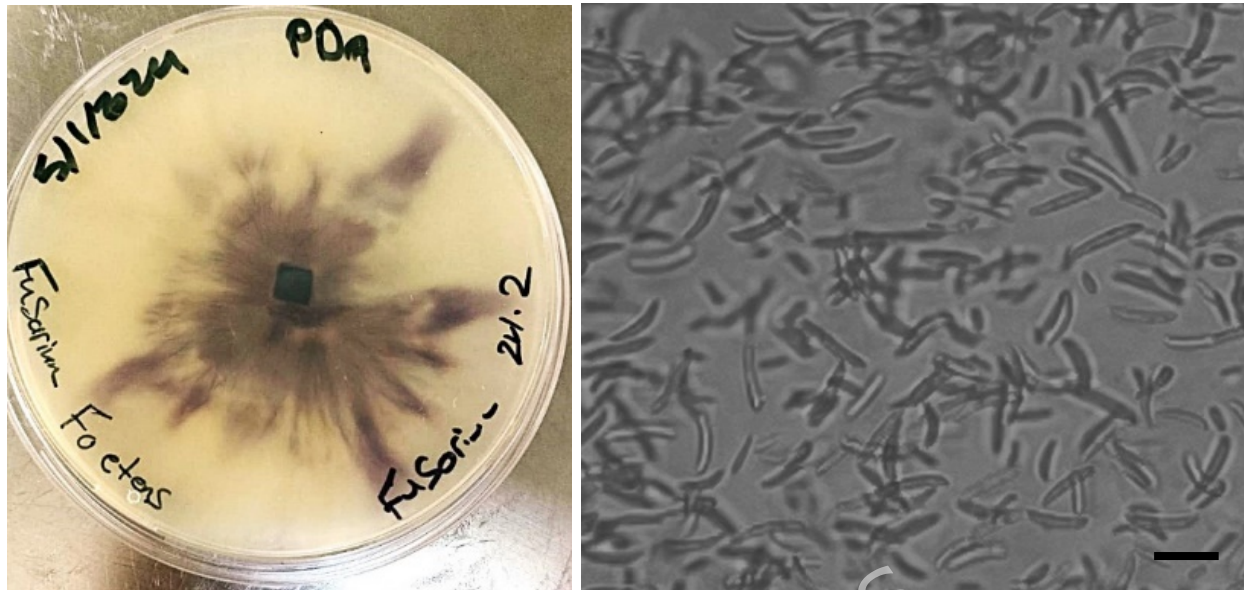
\*Positive control: 3% chlorine (n = 3).  $\Delta$  vs control = mean(treatment) – mean(control). %Max =  $100 \times \text{mean}/40$ , where 40 mm is the maximum measurable diameter.

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## Figures



**Figure 1.** (a) Field inspection in Al-Beqai'a in Tubas district, as shown in the map (generated using Google Maps [41]); (b &c) showed coriander fields where damping-off had occurred.



(a)

(b)



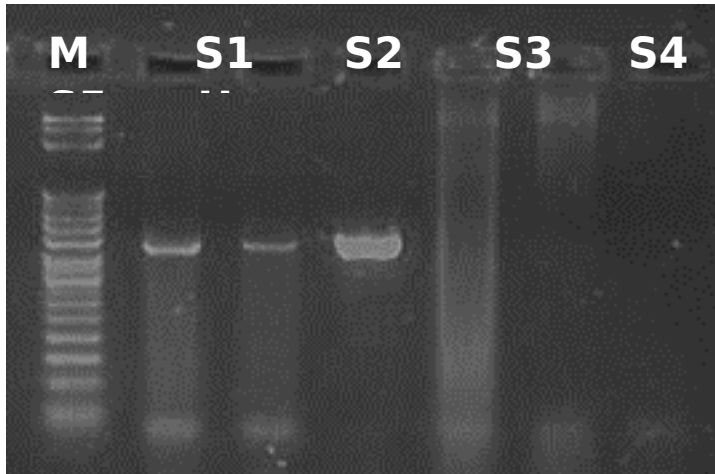
(c)

(d)

**Figure 2.** (a) Colony morphology of the isolated fungus grown on PDA after one week of incubation, showing dense white to pinkish pigmentation characteristic of *Fusarium* spp. (b) Macroconidia observed under the Light

Microscope at 400X typical of *Fusarium* spp. Scale bar =10  $\mu\text{m}$ (c) Koch's postulate test on the coriander seedlings showing thin, dark brown, and water-soaked ("girdling") lesions at the stem near the soil line (d) Healthy non-infected plant.

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**Figure 3.** PCR amplicon of about 550 bp was produced from fungal DNA extracted from samples 1-5 (lanes S) using ITS1 and ITS4 primers Lane M represents the 50 bp DNA ladder (50 bp DNA ladder RTU, GeneDireX) was used. The water control is shown in Lane H.

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