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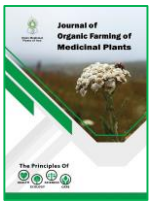

Original Article

First Report of *Fusarium sulawense* Causing Cactus Rot and Its Biocontrol by Essential Oils

Fereshteh Shirzad[a], Hadi Rahanandeh[a], Mahsa Moshayedi[b], Saeid Chavoshi[c]

[a] *Department of Plant protection, Ra.C., Islamic Azad University, Rasht, Iran .*
[b] *Member of the Agricultural Engineering System of Guilan Province.*
[c] *Department of Agronomy and Plant Breeding, Ar.C., Islamic Azad University, Arak, Iran.*

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Corressponding Author:
Hadi Rahanandeh
rahanandeh@iau.ac.ir
rahanandeh20@yahoo.com

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ABSTRACT

Indoor ornamental plants, while enhancing air quality and aesthetics, are highly susceptible to fungal pathogens that compromise plant quality and may indirectly impact human health. In this study, the causal agent of cactus rot in greenhouses of Guilan province,In Iran, was investigated. The pathogen was isolated from symptomatic plants and identified as *Fusarium sulawense* based on morphological and molecular analyses. Given the disadvantages of chemical fungicides, including environmental hazards and the emergence of resistant fungal strains, the antifungal efficacy of 12 plant essential oils was evaluated under laboratory and greenhouse conditions. Essential oils were tested by disc diffusion (four concentrations) and incorporation into culture medium (five concentrations), and their effects were compared with fungicides including Mancozeb, Thiophanate-methyl, Bordeaux mixture, Copper oxychloride, and Rovral-TS at 2000 ppm. Laboratory assays revealed that Ziziphora (*Ziziphora clinopodioides*), Thyme (*Thymus vulgaris*), and Ajwain (*Trachyspermum copticum*) essential oils exhibited the strongest inhibition of mycelial growth. Complete inhibition was observed with Ziziphora clinopodioides, Thymus vulgaris, and Trachyspermum copticum oils, as well as Thiophanate-methyl, Copper oxychloride, and Rovral-TS. In the spore germination assay, Bordeaux mixture and thyme essential oil showed the highest inhibition (100% and 70.67%, respectively). Ajwain, Thyme, Thiophanate-methyl, and Rovral-TS demonstrated fungicidal effects, whereas Ziziphora and Copper oxychloride were fungistatic. Greenhouse trials confirmed that Thyme, Ziziphora, and Ajwain essential oils provided the highest disease control, reducing cactus rot by 85.4%, 83.7%, and 81.9%, respectively, while *Heracleum persicum* (Persian Hogweed) and *Ferula gummosa* (Galbanum) showed negligible activity. Overall, essential oils demonstrated promising antifungal activity, though their efficacy in greenhouse conditions was approximately 30–50% lower than under laboratory conditions.

1. Introduction

Indoor ornamental plants have long been cultivated for enhancing aesthetic appeal and psychological well-being in residential and public environments. They are broadly categorized into flowering and foliage species and are valued not only for their visual attributes but also for their contribution to indoor air quality by modulating humidity, reducing airborne particulates, and mitigating volatile organic compounds (Usman *et al*., 2014; Brilli *et al*., 2018; Li *et al*., 2018). However, these plants are constantly exposed to diverse biotic and abiotic stressors, including pathogenic fungi, which can compromise growth, morphology, and overall physiological performance (Mondal *et al*., 2015).

Cacti, a highly diverse group of succulent plants, are among the most popular indoor ornamentals due to their adaptability to extreme environmental conditions, ranging from arid deserts to humid tropical regions (Williams *et al*., 2014). Taxonomically, cacti belong to three subfamilies—Cactoideae, Opuntioideae, and Pereskioideae—encompassing approximately 100 genera and over 1,500 species (El Mokni *et al*., 2020; Arba *et al*., 2017). This diversity, however, does not preclude their vulnerability to pathogens. Despite their resilience, cacti remain vulnerable to a range of phytopathogens, particularly fungal species such as those within the genus *Fusarium*, which are responsible for rot and vascular wilt diseases (Hyun *et al*., 1998).

The widespread application of chemical fungicides, although effective, has led to the emergence of resistant fungal strains and raised environmental and health concerns, emphasizing the need for sustainable and eco-

friendly disease management strategies. In this context, plant-derived essential oils have emerged as promising biocontrol agents due to their complex mixture of volatile secondary metabolites with potent antimicrobial activities, including antifungal, antibacterial, antiviral, and herbicidal properties (Bakkali *et al*., 2008; Synowiec *et al*., 2017). These compounds act synergistically, reducing the likelihood of resistance development in target pathogens (Jobling, 2000; Tripathi and Dubey, 2004). Recent research has demonstrated the efficacy of essential oils against economically important fungal genera, such as *Fusarium* and *Aspergillus* (Bozik *et al*., 2017).

The present study aims to (i) One of the main objectives of this study was to report, for the first time, the isolation and identification of the causal agent of cactus rot in greenhouses of central Guilan province, Iran., (ii evaluate the antifungal potential of selected plant essential oils at multiple concentrations, and (iii) determine whether these oils exhibit fungistatic or fungicidal activity, thereby providing a basis for environmentally sustainable management of cactus pathogens.

2. Materials and Methods

2.1 Plant Materials and Essential Oils

In this study, essential oils from twelve medicinal plants were used: Eucalyptus (*Eucalyptus globulus* Labill*)*, thyme (*Thymus vulgaris* L.), Ziziphora (*Ziziphora clinopodioides* Lam), peppermint (*Mentha piperita* L.), rosemary (*Rosmarinus officinalis* Spenn), fennel (*Foeniculum vulgare* Mill), myrtle (*Myrtus communis* L.), galbanum (*Ferula gummosa* Boiss), Persian hogweed (*Heracleum persicum* Desf. ex Fisch), lavender (*Lavandula angustifolia* Mill), lemongrass (*Cymbopogon citratus* DC.), and ajwain (*Trachyspermum copticum* L.).

2.2 Fungal Isolation and Culture Conditions

Cactus(*Echinocactus platyacanthus*) 15 samples exhibiting rot symptoms were collected from greenhouses in Rostamabad, Guilan province. Tissue sections of approximately 2–3 cm were excised from the junction of healthy and diseased areas, surface-sterilized in 1% Sodium hypochlorite solution for 60 seconds, and rinsed with sterile distilled water for 60 seconds (Wu et al., 2024). The samples were then incubated at room temperature on potato dextrose agar (PDA). Each emerging fungal colony was subcultured several times to obtain pure cultures, which were stored at 4°C. Pathogenic isolates were identified as causal agents based on their ability to reproduce disease symptoms. Morphological (For example colony characteristics on PDA and microscopic features of macroconidia, such as shape, size, and septation) identification of the isolates was performed using standard Fusarium species identification keys (Nelson *et al*., 1983; Aoki *et al*., 2012).

2.3 ITS-Based Molecular Identification of Fungal Isolates

Molecular identification. Selected fungal isolates were identified using the universal primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAG-3′) to amplify the nuclear rDNA ITS region (Zhou et al., 2022). Genomic DNA was extracted from fresh fungal colonies with the DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's instructions. DNA quality and quantity were assessed with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific) and by 1% agarose gel electrophoresis. PCRs were performed in a final volume of 25 µL containing 12.5 µL of 2× Master Mix (Amplicon, Denmark), 1 µL of each primer (10 µM), 2 µL of template DNA (50 ng/µL), and nuclease-free water to volume. The thermal profile consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 7 min. PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) and Sanger sequenced by Macrogen (Korea). The resulting sequences were edited in BioEdit v7.2 and Chromas v2.6, and compared with GenBank entries using BLASTn.

2.4 Preparation of Plant Essential Oils

Essential oils from the selected plants were extracted using a Clevenger-type apparatus. Briefly, 50 g of dried plant tissues were thoroughly ground and placed separately in a 1-liter round-bottom flask with an appropriate amount of water. The essential oils were extracted by hydro-distillation for 3 hours. Anhydrous sodium sulfate was used to remove residual water from the oils, and the purified essential oils were stored at 4°C until further use (Ranjbar *et al*., 2008).

2.5 Chemical Fungicides

To compare the antifungal activity of plant essential oils, the following chemical fungicides were used: Mancozeb (wettable powder, 80%), Thiophanate-methyl (wettable powder, 70%), Copper oxychloride (wettable powder, 35%), Iprodione + Carbendazim (Rovral, wettable powder, 52.5%), and Bordeaux mixture (suspension, 20%). All fungicides were applied at a final concentration of 2,000 ppm active ingredient by incorporation into the culture medium. This concentration (2,000 ppm a.i.) is the commonly recommended rate for most fungicides and was therefore adopted as the standard for all treatments.

2.6 Assessment of Fungal Growth Inhibition Using the Disc Diffusion Method

To evaluate the inhibitory effects of the selected plant essential oils, sterilized PDA medium was cooled to approximately 45°C and poured into 10-cm Petri dishes. After the medium solidified, a 6-mm fungal disc was excised from the margin of a one-week-old culture and placed at the center of each Petri dish. Plates were incubated at 25°C until the fungal colony reached a diameter of 3 cm (approximately 72 hours) (Hadian *et al*., 2006)..

Sterile 6-mm paper discs were then placed 10 mm from the edge of the colony, and 10 µL of each essential oil concentration was applied onto the discs using a micropipette. The experiment was arranged as a factorial completely randomized design (CRD) with three replicates for each treatment. The factors included plant species and essential oil concentration (1, 10, 100, 1,000, and 2,000 ppm). Colony diameters were measured from the top, left, and right sides at different time intervals over a period of 5 days. Sterile distilled water served as a control to minimize experimental errors (Hadian *et al*., 2006).

2.6 Assessment of Fungal Growth Inhibition by Essential Oils and Fungicides

The inhibitory effects of plant essential oils and fungicides on *F. sulawense* were evaluated using the medium incorporation method. PDA medium was autoclaved, cooled to 45 °C, and amended with essential oils at 1, 10, 100, and 1000 ppm or fungicides at 2000 ppm. Sterile 6 mm mycelial plugs from the colony margin were placed at the center of Petri dishes. Plates were incubated at 25 °C for five days, with three replicates per treatment. Sterile distilled water was used in controls (Hadian *et al.*, 2006).

Growth inhibition (%) was calculated as:.

$$\mathrm{MGI} = \frac{(\mathrm{DC} - \mathrm{DT})}{\mathrm{DC}} \times 100$$

where DC and DT are colony diameters in control and treated plates, respectively (Moslem & El-Kholie, 2009).

2.7 Determination of MIC and MFC of Essential Oils and Fungicides

The minimum inhibitory concentration (MIC) of the tested essential oils and fungicides against *Fusarium sulawense* was determined by incorporating different concentrations into PDA. The lowest concentration completely inhibiting fungal growth was recorded as the MIC (Plodpai *et al*., 2013). For the minimum fungicidal concentration (MFC), samples showing no growth at 2000 ppm above the MIC were sub-cultured onto fresh PDA without test compounds. The lowest concentration causing complete mycelial death, with no regrowth, was considered the MFC (Irkin & Korukluoglu, 2007).

2.8 Effect of Treatments on Spore Germination

The impact of essential oils and fungicides on *Fusarium sulawense* spore germination was assessed using PDA plates containing test compounds at predetermined concentrations. Aliquots of spore suspension (1×10^5)were placed in three spots per plate, each covered with a coverslip, providing three replicates per treatment. Plates were incubated at 25 °C for 24 h. After incubation, the medium beneath each coverslip was excised and observed under a light microscope at 40× magnification. Spores with germ tubes longer than their diameter were counted as germinated, and the percentage of germination inhibition was calculated analogous to mycelial growth inhibition (Tatsadjieu *et al*., 2009). The experiment followed a completely randomized design with three replicates. Sterile distilled water was used in control plates, and data were analyzed using Duncan's multiple range test at 5% significance.

2.9 Greenhouse Evaluation of Essential Oils on E. platyacanthus

A completely randomized design with 14 treatments—including 12 essential oils (thyme, savory, ajwain, eucalyptus, lavender, fennel, peppermint, lemongrass, marjoram, rosemary, artemisia, and golpar) and two controls (healthy and infected)—was implemented with three replicates per treatment. Uniform *E. platyacanthus* plants were grown in 15 cm pots containing sterilized soil (garden soil:sand:peat moss, 2:1:1) (El-Komy et al., 2015).

The pathogenic fungus was cultured on the surface of a Petri dish containing PDA medium, and after seven days the surface of the Petri dish was washed with sterile distilled water to prepare a spore suspension. Plants were inoculated with *F. sulawense* at 5×10^6 spores/mL (10 mL per plant, crown injection). Twenty-four hours later, essential oils were applied as 2000 ppm aqueous solutions with Tween 80 (10 mL per pot) weekly for four weeks (Abd-El-Kareem *et al*., 2019). Greenhouse conditions were 28 ± 2 °C day/20 ± 2 °C night, 60–70% relative humidity, and controlled natural light. After 40 days, disease incidence (DI), disease severity index (DSI), and fresh and dry weights of aerial parts were recorded (Kumar et al., 2020).

$$\text{Disease Incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}} \times 100$$

$$\text{DSI (\%)} = \frac{(\text{Number of plants at each rating} \times \text{Disease severity rating})\Sigma}{\text{Total number of plants} \times \text{Highest severity class}} \times 100$$

using a 0–4scale: 0 = healthy, 1 = mild symptoms, 2 = <25% root infection, 3 = 25–50% root infection, 4 = >50% infection or plant death.

2.10 Statistical Analysis

All data were subjected to ANOVA based on a factorial arrangement in a completely randomized design using SAS version 9.4. Mean comparisons were performed

using Duncan's multiple range test. Graphs were generated in Microsoft Excel.

3. Results

3.1 Pathogenicity of Fungal Isolates

In cactus production greenhouses, symptoms of crown rot were observed at the plant collar region. Twelve fungal isolates were recovered, of which three isolates (CR1, CR2, and CR3) were the most frequently observed. Based on morphological characteristics, the obtained isolates were identified as *Fusarium sulawense*. Colonies grown on PDA appeared cottony and floccose, initially white to cream in color, gradually turning pale pink after several days. Microconidia were mostly single-celled, ovoid to ellipsoidal, formed in chains on short phialides, and measured approximately 5–10 × 2–3 µm**.** Macroconidia were slender, falcate, and typically contained 3–5 septa, with lengths ranging from 20–35 µm and widths of 3–4 µm. Thick-walled chlamydospores were observed either singly or in pairs, intercalary or terminal within hyphae. Conidiogenous structures were sporodochia consisting of phialides borne on short branched conidiophores. The sporodochia appeared as compact, cushion-shaped to globose masses on the colony surface, measuring approximately 150–400 µm in diameter. ITS sequences of CR1, CR2, and CR3 showed 100% identity to reference strains of *F. sulawense* LC7919 and LC7920 (GenBank accession numbers MK280811 and MK280805) previously isolated from China. Following inoculation of these isolates on to *Echinocactus platyacanthus*, disease symptoms were visible after 20 days.

3.2 Effect of Plant Essential Oils on Fungal Growth (Disk Diffusion Method)

The inhibitory effects of the tested essential oils varied significantly among oils and concentrations (Figure 1). Except for marjoram and lemongrass oils, which showed no inhibitory effect even at the highest concentration, all other oils exhibited antifungal activity. Based on the average percentage of growth inhibition, savory, ajwain, and thyme demonstrated the highest inhibition of *F. sulawense* mycelial growth at 44.10%, 62.8%, and 45.8%, respectively (Fig 1).

3.3 Effect of Plant Essential Oils and Fungicides on the Growth of the Causal Fungus Using the Medium Incorporation Method

All tested plant essential oils significantly inhibited the mycelial growth of *F. sulawense.*

The strongest inhibitory effect was observed at 2000 ppm for Zataria, Thyme, and Ajwain, which completely suppressed mycelial growth.

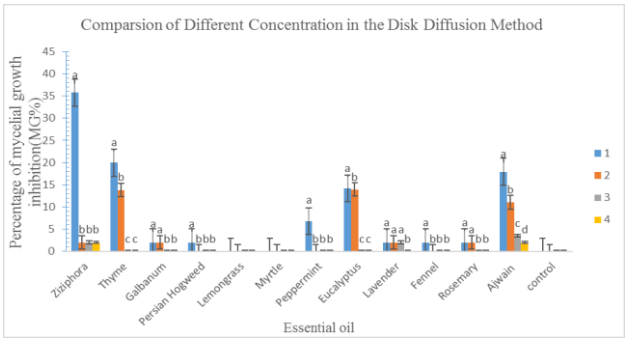

Fig 1. Effect of different concentrations of plant essential oils on *Fusarium sulawense* growth using the disk diffusion method.

Other essential oils, including Lavender, Eucalyptus, and Fennel, also exhibited notable inhibition, reducing mycelial growth by 96.76%, 75.9%, and 75%, respectively (Fig 2). A significant difference was observed between the first(2000ppm) and second (1000ppm) concentrations for all essential oils. Statistical analysis indicated that most concentrations of each essential oil fell into three distinct groups, except for Ajwain, where four concentrations were assigned to four separate statistical groups. These results highlight the strong and concentration-dependent effects of the essential oils on the mycelial growth of *F. sulawense*.

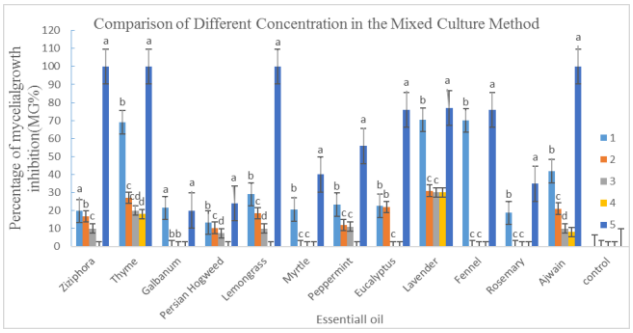

Fig 2. Mycelial growth inhibition of *F. sulawense* by different concentrations of plant essential oils using the culture media mixing method.

Based on statistical analysis, all tested fungicides effectively reduced the mycelial growth of *F. sulawense*. The strongest inhibitory effects were observed with Copper Oxychloride, Roral TS, and Thiophanate-methyl, which completely suppressed mycelial growth. In contrast, the lowest inhibitory effects were recorded for Bordeaux mixture (Cu-based) and Mancozeb. These results highlight the varying efficacy of chemical fungicides against the mycelial development of *F. sulawense*(Fig 3).

3.4 Effect of Plant Essential Oils and Chemical Fungicides on the Spore Germination of F. sulawense

Analysis of variance showed that the type of essential oil significantly affected the inhibition of *F. sulawense* spore germination ($p \leq 0.01$).

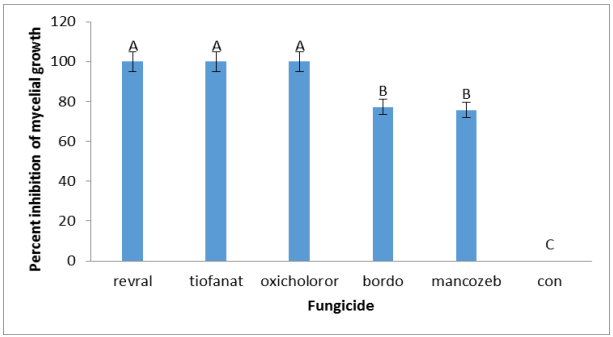

Fig 3. Mean percentage inhibition of *F. sulawense* spore germination by five fungicides. Different letters denote significant differences at $p \leq 0.05$.

Thyme essential oil exhibited the highest inhibition (67.70%), followed by Zataria (65.66%) and Ajwain (67.50%), which formed the next statistically distinct group. Essential oils of Barijeh, Lemongrass, Fennel, and Marjoram showed no significant effect compared to the control (Table 1). Among chemical fungicides, Bordeaux mixture showed the strongest inhibition, whereas Copper Oxychloride was statistically similar to the control. In the case of Mancozeb, the culture medium turned completely yellow after its addition, which prevented the evaluation of spore germination; thus, no results could be recorded.

Table 1. Mean inhibition of *F. sulawense* spore germination by 2000 ppm of plant essential oils and fungicides.

Inhibition percentage		Material
50.67^{c}	Ajwain	
0^{f}	Galbanum	
12^{e}	Persian Hogweed	
70.67^{b}	Thyme	
0^{f}	Lemongrass	
0^{f}	Myrtle	
24^{d}	Peppermint	Essential oil
22.67^{d}	Eucalyptus	
20^{d}	Lavender	
0^{f}	Fennel	
22.34^{d}	Rosemary	
66.65^{c}	Ziziphora	
100^{a}	Bordeaux mixture	
84^{b}	Revral-TS	
52^{c}	Thiophanate-methyl	Fungicide
-	Mancozeb	
5.33^{e}	Copper Oxychloride	
0^{f}		control

3.5 Fungicidal and Fungistatic Effects of Essential Oils and Fungicides on the Target Fungus

Treatments in which no fungal growth was observed were re-cultured on PDA medium without essential oils, and after 7 days the presence or absence of growth was recorded in Table 3. According to the results, the essential oils of Ajwain and Thyme, along with the fungicides Thiophanate-methyl and Roral TS (Fig 4), exhibited fungicidal effects, whereas Zataria essential oil and Copper Oxychloride showed fungstatic activity(Table 2).

Table 2. Fungicidal and fungistatic effects of 2000 ppm essential oils and fungicides on *F. sulawense*.

F. sulawense	Essential oil
FC	Ajwain
FC	Thyme
FS	Zataria
FC	Thiophanate-methyl
FS	Copper Oxychloride
FC	Rovral-TS

FS:Fungistat , FC:Fungicide

Fig 4. From right to left: essential oils of ajwain (*Trachyspermum ammi*), thyme (*Thymus vulgaris*) and the fungicides Thiophanate-methyl at 2,000 ppm, plus the control, assayed against *F. Sulawense*

3.6 Effect of Plant Essential Oils on Disease Reduction in Greenhouse Conditions

The results of this study showed that plant essential oils had differential efficacy in controlling cactus disease. Thyme, Zataria, and Ajwain essential oils exhibited the highest disease control percentages (84.5%, 83.7%, and 82.9%, respectively). These treatments also significantly increased plant growth, with dry weight reaching approximately 4 g and fresh weight around 7.7 g, and were grouped statistically in category “a.” Eucalyptus, Lavender, and Fennel essential oils showed moderate effects, with disease control ranging from 40% to 48%, and were placed in group “b.” Lemongrass essential oil had a weaker effect, providing only 22% disease control, and fell into group “c.” Essential oils of Myrtle, Rosemary, Barijeh, and Heracleum had minimal impact on disease control, with inhibition percentages close to zero and dry/fresh weights similar to the infected control, and were classified in group “e” (Table 4).

4. Discussion

Based on morphological and molecular analyses, the fungus isolated from cactus in this study was identified as *F. sulawense*. *Fusarium sulawense* is a newly described species of Fusarium, first identified in 2019 (Xia *et al*., 2019; Maryani et al., 2019). It was initially isolated from banana in Indonesia in 2014 and has been reported as an endemic species of this crop (Maryani *et al*., 2019).

This species belongs to the *F. incarnatum-equiseti* species complex, which comprises more than 30 pathogenic species (Jedidi *et al.*, 2012). *F. sulawense* has

Table 3. Effect of plant essential oils on disease control and growth of cactus under greenhouse conditions.

Plant fresh weight (g)	Plant dry weight (g)	Disease control (%)	Plant essential oil
22.0±1.2a	6.7±0.4a	100a	Healthy control
9.0±0.6d	2.1±0.2d	0d	Infected control
۲۱,۴±۱,۱a	6.5±0.4a	85.43±3.2a	Thyme
۲۰,۹±۱,۲a	6.3±0.3a	83.7± 2.6a	Zataria
۲۰,۲±۱,۰a	6.0±0.3a	81.9±2.8a	Ajwain
۱۵,۳±۰,۹bc	4.5±0.3bc	47.8±2.1bc	Eucalyptus
۱۴,۸±۰,۹bc	4.3±0.2bc	44.5±2.0bc	Lavender
۱۴,۲±۰,۷bc	4.1±0.3bc	42.2±2.5bc	Fennel
۱۲,۶±۰,۶cd	3.5±0.2cd	22.3±1.9cd	Lemongrass
۱۲,۲±۰,۵cd	3.3±0.2cd	19.8±2.2cd	Myrtle
۱۱,۹±۰,۷cd	3.1±0.2cd	18.1±2.0cd	Rosemary
۹,۴±۰,۵d	2.4±0.2d	4.2±1.5d	Barijeh
۹,۲±۰,۴d	2.3±0.2d	3.7±1.3d	Heracleum
۱۷,۵±۰,۸b	5.2±0.3b	58.5±2.4b	Peppermint

Values represent the mean percentage of disease control, fresh weight, and dry weight of cactus plants treated with different essential oils. Means followed by the same letter within each column are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

been shown to infect a wide range of crops in tropical and subtropical regions. For instance, it caused melon fruit rot in Brazil in 2020 (Lima *et al*., 2021) and has also been associated with papaya fruit rot, mango leaf spot, and plum blight in China (Yi *et al.*, 2022). Additionally, it has been identified as the causal agent of disease in mahogany (*Swietenia macrophylla*) and wheat head blight in Mexico (Leyva-Mir *et al.*, 2022). In the Fars province of Iran, *Fusarium oxysporum* f. sp. *opuntiarum* has been previously reported as the causal agent of cactus rot (Safaei Farahani & Mostofi-Zadeh, 2015).

The repeated and excessive use of chemical compounds not only leads to environmental pollution but also induces resistance in pests, thereby significantly increasing the potential damage caused by pathogenic agents (Narayanasamy, 2002). Currently, plant-derived compounds have been commercially developed for plant disease management. Examples include Neemazol, derived from *Azadirachta indica*, used to control powdery mildew in chickpea caused by *Erysiphe pisi*, and Milsana, obtained from *Reynoutria sachalinensis*, used to manage *Leveillula taurica* (Konstantinidou-Doltsinis *et al*., 2006). Plant essential oils can be integrated into integrated pest management (IPM) systems and organic crop production. Due to their complex and mostly volatile composition, they are capable of controlling plant diseases. Many essential oils and their constituents exhibit antifungal properties; however, their direct application in controlling plant and animal fungal diseases is limited by high production costs and low concentrations of active compounds (Alam *et al*., 2025).

Our laboratory assays revealed that the antifungal efficacy of essential oils against *F. sulawense* was highly dependent on the plant source, with oils from the Lamiaceae and Apiaceae families showing the greatest promise. In this study, the effects of essential oils from Heracleum persicum, Lemongrass, Rosemary, Lavender, Myrtle, Fennel, Eucalyptus, Thyme, Zataria, Barijeh, Peppermint, and Ajwain on *F. sulawense*, the causal agent of cactus disease, were evaluated under laboratory conditions. Thymol and carvacrol are phenolic terpenoid compounds that exhibit the highest antimicrobial activity compared to other active constituents, such as terpenes and terpenoids with other phenolic groups (Abdel-Kader *et al.*, 2011). Carvacrol has been reported to exert stronger inhibitory effects than thymol (Gonzalez et al., 2024). Our results corroborate earlier findings on the broad-spectrum efficacy of oils from the Lamiaceae family, such as Thyme.

Essential oils from Thyme, Lavender, and Summer savory are capable of inhibiting the growth of fungi affecting food, horticultural, and field crops, and could serve as alternatives to current chemical fungicides (Rasooli *et al.*, 2009). The disc diffusion assay revealed that Zataria essential oil exerted the most pronounced inhibitory effect on the mycelial growth of F. sulawense, whereas Thyme and Ajwain exhibited comparatively lower activity. These observations underscore the differential sensitivity of the pathogen to various essential oils and concentrations, and emphasize that their biocontrol potential is intrinsically linked to chemical composition, with dominant constituents serving as key determinants of antifungal efficacy. (Plotto *et al.*, 2003). Thymol, present in Zataria, Thyme, and Ajwain, is one of the major active compounds. Previous studies have shown that essential oils from these three plants exert strong inhibitory effects on the mycelial growth of *F. oxysporum*, with Thyme and Zataria completely preventing fungal growth (Lotfi *et al.*, 2015). Among plant essential oils, Thyme is considered one of the most effective against phytopathogenic fungi and bacteria (Mohammadpour *et al*., 2015).

Trachyspermum ammi L. (Ajwain) has confirmed antifungal activity. The antifungal effects of Thyme, Summer savory, and Ajwain on *Alternaria solani* in tomato demonstrated that Ajwain essential oil could effectively replace chemical fungicides. According to the results of the present study, the most effective essential oils against *F. sulawense* were Zataria, Thyme, and Ajwain, which completely inhibited the pathogen in the culture medium assay. Similarly, previous studies

reported that Thyme and Ajwain essential oils completely inhibited the growth of *F. graminearum* and *F. solani* in culture medium assays (Izadi *et al*., 2025).

Essential oils from Ajwain, Thyme, Zataria, and Summer savory also exhibited strong inhibition of conidial germination in *B. cinerea*. These oils contain bioactive compounds such as carvacrol, thymol, trans-anethole, γ-terpinene, and p-cymene, which are likely responsible for their antifungal properties (Siripornvisal *et al*., 2009). Essential oils from Eucalyptus, Lavender, and Fennel showed over 75% inhibition of conidial germination in *F. sulawense*. In other species such as *F. solani* and *F. oxysporum*, these oils exhibited moderate inhibitory effects (Davari & Ezazi, 2022).

The present study demonstrated that both the type of essential oil and its applied concentration significantly influenced the inhibition of spore germination. Among the tested oils, Ajwain, Thyme, and Zataria showed the highest inhibitory effects on fungal spore germination. Essential oils are also characterized by low EC50 values, high efficacy, strong antimicrobial properties, and the ability to reduce disease incidence (Bi *et al*., 2012). Variations in reported antimicrobial activity are often attributed to differences in chemical composition and fungal isolates (Reedy e*t al*., 2008). The biodegradability of plant extracts and essential oils, their low toxicity to humans and other mammals, and their minimal environmental impact make these compounds suitable alternatives or complements to chemical fungicides for crop protection and post-harvest storage (Masson *et al*., 2013).

Fungicide assays were conducted to evaluate both mycelial growth and spore germination inhibition. In spore germination tests, Bordeaux mixture exhibited the highest inhibition, whereas Copper Oxychloride had the lowest effect. Except for Thiophanate-methyl and Mancozeb, the other three fungicides exhibited either fungicidal or fungistatic activity. Among essential oils, Zataria demonstrated fungistatic effects. In other genera such as *Aspergillus* and *Sclerotinia*, Zataria has also shown fungistatic and fungicidal activity (Mazarei & Rafati, 2019). Ajwain and Thyme completely destroyed fungal mycelia, likely due to their high content of carvacrol and thymol, which are known to inhibit mycelial growth (Purkait *et al*., 2020). These oils were comparable to commonly used greenhouse fungicides such as Rovral-TS and Thiophanate-methyl, widely applied in ornamental greenhouses in the Guilan and Mazandaran provinces.

Greenhouse experiments indicated significant differences among essential oils in controlling fungal disease on *Echinocactus platyacanthus*, as well as in improving plant fresh and dry weights and growth indices. Thyme, Zataria, and Ajwain were the most effective, achieving disease control rates of 85.4%, 83.6%, and 81.2%, respectively. Their high efficacy is likely attributable to the presence of strong bioactive antifungal compounds (Burt, 2004; Raveau *et al*., 2020). In contrast, Eucalyptus, Lavender, and Fennel exhibited less than 50% disease control, possibly due to rapid volatilization, low stability under greenhouse conditions, and limited penetration into plant tissues (Raveau et al., 2020; Regnault-Roger *et al*., 2012). Essential oils from Heracleum, Barijeh, Rosemary, and Myrtle showed minimal antifungal effects, with plant fresh and dry weights similar to the infected control, indicating low efficacy under practical conditions. In addition to phenolic compounds, other factors such as degradation by phylloplane microorganisms, enzymatic activity, pH, formulation type, and application method (e.g., spray or encapsulated) influence the final efficacy of essential oils (Regnault-Roger *et al*., 2012; Bakkali et al., 2008). Therefore, selecting appropriate formulations to enhance stability and controlled release remains a major challenge in the practical application of essential oils.

5. Conclusion

Based on the results of this study, essential oils from Thyme, Ajwain, and Kakuti can be effectively used alongside chemical fungicides to control the cactus pathogenic fungus, particularly in household settings, provided that suitable formulations are employed to enhance stability and efficacy. In contrast, other essential oils with lower efficacy may require optimization or combination with other antifungal agents to improve their performance. Plants containing higher amounts of carvacrol and thymol, as reported in previous studies, exhibited stronger inhibition against the fungus in all experiments. The present findings not only enhance our understanding of the environmental impact on bioactive compounds but also highlight new avenues for practical applications and further studies.

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