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Evaluation of the Antifungal Activity of Aqueous Plant Extracts Against Fusarium oxysporum f. sp. lycopersici

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A B S T R A C T

This study examined the antifungal activity of cold aqueous extracts from ten locally available plant species in Libya against *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of tomato wilt disease. The pathogen was isolated from infected tomato plants collected in the Ain Zara region and identified through cultural and microscopic characteristics, with confirmation using Komada selective medium. Plant materials were extracted by cold maceration, and the resulting solutions were tested using the poisoned food technique at concentrations of 20, 40, 60, 80, and 100%.

Clear differences were recorded among the plant extracts in their ability to inhibit fungal growth. Clove extract was the most effective, achieving complete inhibition at concentrations of 40% and above. Garlic, thyme, and pomegranate peel also showed strong antifungal activity, with garlic producing full inhibition at 80–100% and thyme at concentrations of 60% and higher. In contrast, extracts of wormwood (*Artemisia herba-alba*) and oleander (*Nerium oleander*) caused minimal inhibition even at maximum concentration.

Microscopic observations revealed that the most active extracts significantly reduced the formation of macroconidia and microconidia after 14 days, while chlamydospores remained less affected. Preliminary phytochemical screening indicated that the highly active extracts contained phenolics, flavonoids, terpenoids, tannins, and alkaloids, compounds known to disrupt fungal structures and interfere with essential metabolic processes.

These findings demonstrate that aqueous plant extracts—particularly clove, garlic, thyme, and pomegranate—offer promising, environmentally friendly alternatives to synthetic fungicides. Further greenhouse and field studies are recommended to evaluate their applicability within sustainable disease management programs for tomato production in Libya.

Introduction

Tomato (*Lycopersicon esculentum* L.) is a major crop in the Solanaceae family, valued for its nutritional richness and global dietary significance (Sainju, 2003; Fritz, 2005). It contains essential amino acids, vitamins, minerals, carbohydrates, proteins, and fats, contributing to food security worldwide. Ranked second after potato in cultivated area, tomato production reaches about 192 million tonnes from 5 million hectares globally, including 230,000 tonnes from 11,000 hectares in Libya (FAO, 2023).

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici*, is among the most destructive diseases of tomato crops throughout all growth stages, especially in susceptible cultivars under conducive environmental conditions (Abughanya, 1998; Diab, 2018).

The pathogen's ability to survive in soil for long periods via chlamydospores and its production of phytotoxic compounds that block xylem water transport exacerbates control difficulties (Jahanshir & Dzhalilov, 2010; Shanmugam et al., 2015). Chemical control remains largely ineffective due to poor vascular penetration and leads to environmental contamination,

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the development of resistant fungal strains, and adverse effects on human health (Komarek et al., 2010; Hahn, 2014).

Libya's diverse flora presents an opportunity to explore plant-based solutions for controlling phytopathogens as part of sustainable agriculture systems (Talibi et al., 2012; El-Gamal & Mahmoud, 2019). Several studies have demonstrated the efficacy of garlic (*Allium sativum*), ginger (*Zingiber officinale*), eucalyptus (*Eucalyptus* spp.), and mint (*Mentha* spp.) extracts against *F. oxysporum*, inhibiting growth and spore formation (Katooli et al., 2011; El-Mohamedy & Abdelah, 2014; Omer et al., 2023).

As chemical fungicides prove increasingly inefficient, expensive, and environmentally detrimental, the search for natural and eco-friendly alternatives has gained momentum. Among these, plant extracts have shown antifungal potential without harming the environment or human health (Javaid & Iqbal, 2014; Abd-ElGawad et al., 2023). Their antifungal efficacy is attributed to bioactive compounds such as phenolics, flavonoids, terpenes, volatile oils, and alkaloids, which disrupt fungal membranes and inhibit growth and sporulation (Cowan, 1999; Dixon et al., 1983; Rongai et al., 2012).

However, studies focusing on aqueous extracts of native Libyan plants remain limited, necessitating further investigation into their antifungal potential and the identification of active chemical constituents, especially given the abundance of medicinal plants in Libya rich in antifungal compounds such as glucosinolates, phenolics, and volatile oils (Jeewon et al., 2024).This study aimed to evaluate the inhibitory activity of aqueous extracts from selected local plants against Fusarium oxysporum f. sp. lycopersici under laboratory conditions.and Conduct a preliminary phytochemical screening to identify potential active constituents within these extracts.

Materials and Methods

Study Location and General Framework

The study was conducted during the 2024–2025 growing season at the Plant Pathology Laboratory, Department of Plant Protection, and the Soil, Water, and Plant Analysis Laboratory, Department of Soil and Water Sciences, Faculty of Agriculture, University of Tripoli, Libya. All experimental and analytical procedures were performed under aseptic conditions to ensure accuracy and reproducibility.

Laboratory Equipment and Materials

Instruments

The principal laboratory instruments used included an autoclave, incubator, electric oven, hot plate with magnetic stirrer, pH meter (Hanna Instruments, Italy), electric blender, shaker, vacuum pump, compound light microscope (Olympus CX23, Japan), sensitive balance, Bunsen burner, 5-mm cork borer, and a laminar flow hood. All procedures followed standard phytopathological methodologies described by Dhingra and Sinclair (1985).

Chemicals

Analytical-grade chemicals were employed for fungal isolation, preparation of plant extracts, and phytochemical screening. These included sodium hypochlorite, ethanol (95%), potassium iodide, phenol crystals, lactic acid, ferric chloride, and chloroform. All reagents were procured from Sigma-Aldrich (Germany) and used according to the protocols outlined by Harborne (1984) and Shihata (1951).

Culture Media

Two culture media were utilized throughout the study:

Potato Dextrose Agar (PDA): Prepared by dissolving 39 g of commercial PDA powder in 1 L of distilled water, followed by sterilisation at 121°C and 15 psi for 20 minutes. PDA was used for the isolation, purification, and growth assessment of *Fusarium oxysporum*.

Komada Medium: This selective medium was used for the confirmation of *F. oxysporum* based on its characteristic colony morphology and pigmentation. The medium was formulated according to Komada (1975) and later modifications by Rowe et al. (1980), which suppress non-target fungal contaminants to allow precise identification of *Fusarium* spp.

Isolation and Identification of the Pathogenic Fungus *Fusarium spp.*

Infected tomato plants showing typical symptoms of Fusarium wilt and internal anatomical signs were collected from greenhouses in the Ain Zara region. The stems and roots were washed with water to remove soil particles, and the infected parts were cut into small pieces. These were surface-sterilized with a 1% sodium hypochlorite (NaOCl) solution for two minutes, rinsed again with sterile distilled water, and dried on sterile filter paper. The sterilized tissue segments were then transferred to Petri dishes (9 mm in diameter) containing potato dextrose agar (PDA) medium, with four pieces per dish. The plates were incubated for five days at 25 ± 2 °C. After the fungal mycelium developed, pure cultures were obtained using the single spore isolation technique on PDA medium.

The pathogenic fungus was identified based on its morphological and microscopic characteristics, including colony color, pigment production in the medium, the shape and size of the sickle-shaped conidia, and the number of cells per spore. Identification followed the taxonomic keys and criteria described by Dhingra et al. (1985), Abughania and

Elarabi (1998), Wong (2003), Summerell et al. (2003), and Ignjatov et al. (2012).

On PDA medium, the fungus produced pale white mycelium, which turned pink to purple as conidia formed. Microscopic examination of fungal growth revealed both small conidia (microconidia) and large conidia (macroconidia). The fungus was identified as *Fusarium oxysporum*. Pure fungal cultures were maintained at 4 °C in a refrigerator until use.

The Komada selective medium was used to confirm the identification of *Fusarium spp.* as *F. oxysporum*. The medium was prepared in two parts: the first part was mixed with continuous stirring until boiling and sterilized in an autoclave. The second part was prepared by dissolving all components except chloramphenicol, which was added after sterilization. The two parts were combined when the temperature of the first part reached 45 °C, then poured into 9 cm Petri dishes. Discs of *Fusarium spp.* isolates were inoculated onto both Komada and PDA media. The average radial growth of the fungal colonies was measured in two directions daily and compared with the PDA control to assess the effect of each medium on the growth and development of *Fusarium* spp. colonies.

A 5 mm disc from a pure culture of *Fusarium* spp. was placed in the center of a 9 cm Petri dish containing either PDA or Komada medium. Plates were incubated at 25 ± 2 °C, with five replicates per treatment. The mean radial growth of the fungal colonies was measured in two directions daily until the colony in the control (PDA) reached 9 cm in diameter. Colony growth characteristics on Komada medium were compared with those on PDA as a control, observing the effects of the media on the growth and development of *Fusarium* spp. isolates (Molay Al Hassan, 2006; Rowe, 1980; Wong, 2003; Nel et al., 2005).

Plant Material Collection and Preparation

Ten plant species from different botanical families—including (Syzygium aromaticum) , (Thymus vulgaris) , (Allium sativum) , (Punica granatum), (Eucalyptus camaldulensis) , (Mentha spicata) , (Rosmarinus officinalis) , (Origanum majorana) , (Artemisia herba-alba) , (Nerium oleander) were collected from multiple regions across Libya (Tripoli, Gharyan, and Tarhuna). Plant materials such as leaves, buds, and cloves were thoroughly washed, shade-dried at room temperature, and ground into fine powders using a sterile electric grinder. The powders were then stored in sterile glass containers until extraction (Al-Zubairi et al., 2019).

Aqueous Extract Preparation

Aqueous extracts were prepared via cold maceration. Three hundred grams of each dried plant powder were homogenised in 500 mL of sterile distilled water using a blender. The mixture was shaken for 24 hours at 25 ± 2°C in the dark, filtered through double-layered sterile muslin cloth, followed by Whatman No. 1 filter paper using a vacuum pump. The filtrates were further sterilised through a 0.22 µm Millipore filter to remove microbial contaminants. Extracts were stored at 4°C in sterile amber glass bottles until use (Ghoneem et al., 2014).

Antifungal Assay of Plant Extracts

The antifungal efficacy of the plant extracts was evaluated using the poisoned food technique (PFT) as described by Dhingra and Sinclair (1985). PDA was amended with five extract concentrations: 20%, 40%, 60%, 80%, and 100%. Plates were inoculated centrally with a 5-mm mycelial disc obtained from a 7-day-old culture of *F. oxysporum* and incubated at 25 ± 2°C for 14 days. Colony diameters were measured along two perpendicular axes.

The percentage of mycelial growth inhibition was calculated according to Vincent (1947) using the following formula:

$$I=C-T/C \times 100$$

Where:
I = inhibition percentage;
C = colony diameter in control;
T = colony diameter in treatment.

Microscopic examination of hyphal and spore morphology was carried out using lactophenol cotton blue staining to observe structural alterations caused by the plant extracts (Ellis, 1994).

Preliminary Phytochemical Screening

A preliminary chemical screening was conducted for some active compounds in the plant samples. The pH value was first determined by mixing 10 g of the dried plant powder with 50 mL of distilled water using a magnetic hot plate stirrer for 10 minutes. The mixture was then filtered, and the pH value was measured using a pH meter (Shihata, 1951).

Flavonoids were detected by dissolving 10 g of the dried plant powder in 50 mL of 95% ethanol and filtering the solution (A). Then, 10 mL of 50% ethanol was added to 10 mL of 50% potassium hydroxide (KOH) solution (B). When equal volumes of the two solutions were mixed, a yellow color appeared, indicating the presence of flavonoids (Jaffer et al., 1983).

For glycosides detection, 5 mL of the plant extract was added to 2 mL of glacial acetic acid, one drop of ferric chloride ($FeCl_3$) solution, and 1 mL of concentrated sulfuric acid. The appearance of a brown ring at the interface indicated the presence of glycosides (Lee et al., 2016).

Alkaloids were detected by boiling 10 g of the plant powder in 50 mL of distilled water containing 4%

hydrochloric acid. The solution was filtered and cooled, then 1 mL of the filtrate was placed in test tubes and treated with Dragendorff's, Wagner's, and Mayer's reagents prepared according to standard procedures (Harborne, 1984). The formation of an orange precipitate with Dragendorff's reagent, a brown precipitate with Wagner's reagent, and a white or cloudy precipitate with Mayer's reagent indicated the presence of alkaloids.

Saponins were confirmed by adding 3 mL of mercuric chloride solution to 5 mL of the plant extract, where the formation of a white precipitate confirmed their presence (Shihata, 1951). Additionally, a foam test showed a stable, persistent froth lasting for 15 minutes (Szychowski et al., 2018).

Resins were detected by dissolving 5 g of the dried plant powder in 50 mL of 95% ethanol, heating the mixture in a water bath at 100°C for two minutes, and filtering it. Then, 100 mL of distilled water acidified with 4% hydrochloric acid was added. The appearance of turbidity in the solution indicated the presence of resins (Shihata, 1951).

Tannins were detected by dissolving 5 g of the plant powder in 50 mL of distilled water and boiling it. The filtrate was divided into two portions: 1% lead acetate solution was added to the first, and 1% ferric chloride solution to the second. The formation of a white gelatinous precipitate in the first and a yellowish-green coloration in the second indicated the presence of tannins (Shihata, 1951).

Terpenes were detected by adding 1 mL of the plant extract to 2 mL of chloroform, followed by a few drops of acetic anhydride and one drop of concentrated sulfuric acid. The appearance of a brown color confirmed their presence (Harborne, 1984).

Finally, phenols were detected by dissolving 0.1 g of the extract in 1 mL of distilled water and adding 1–2 drops of 1% ferric chloride ($FeCl_3$) solution. The appearance of a blue or green color confirmed the presence of phenols (Gayon, 1972).

Statistical Analysis

The experimental design followed a completely randomised design (CRD) with three replications per treatment. Data were subjected to analysis of variance (ANOVA) using SAS statistical software (Version 9.1; SAS Institute, 2001). Mean separation was performed using Duncan's Multiple Range Test (DMRT) at probability levels of $p \leq 0.05$ and $p \leq 0.01$ (Duncan, 1955).

Results and Discussion

Effect of Plant Extracts on Mycelial Growth and Sporulation

The antifungal activity of the examined plant extracts was evaluated by recording colony diameter and calculating inhibition percentages. The extracts exhibited a concentration-dependent inhibitory effect, where higher doses produced stronger suppression of mycelial growth. Clove extract showed the highest potency, achieving complete inhibition (100%) at concentrations of 40% and above, while at 20% it reduced the colony diameter to 0.70 cm (Figure 1).

Figure 1. Effect of the cold aqueous extract of clove on the growth of *F. oxysporum*.

The strong antifungal effect of thyme is attributed to eugenol and related volatile oils known to disrupt fungal membranes (Hussein, 1981; Chalfoun et al., 2004; Khammass, 2011, 2023).Thyme extract exhibited complete inhibition at concentrations ≥60%, and moderate inhibition at lower doses (Figure 2).

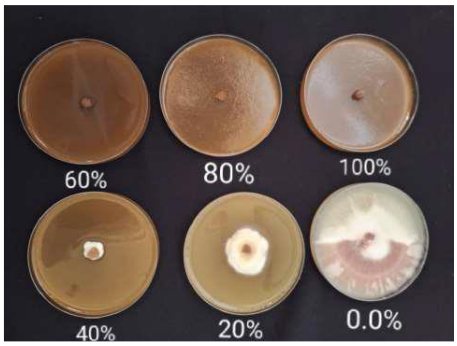

Figure 2. Effect of the cold aqueous extract of thyme on *F.oxysporum* fungus.

Thymol and carvacrol are the principal active compounds responsible for its activity (Sacchetti et al., 2005; Horita & Kodama, 1996).Garlic extract showed strong inhibitory activity, with full inhibition achieved at 80–100% concentrations (Figure 3).

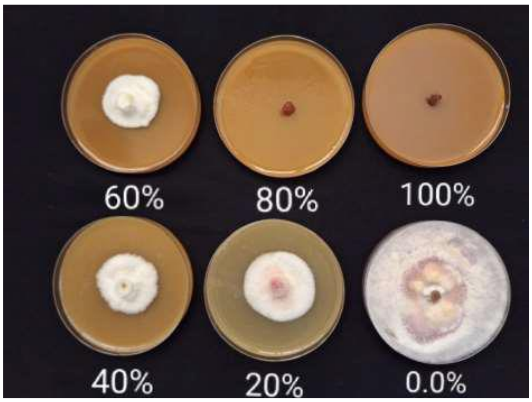

Figure 3. Effect of the cold aqueous extract of garlicon the growth of *F. oxysporum* fungus.

This is consistent with the presence of allicin and sulfur-containing compounds with known antifungal actions (Chunmei et al., 2010 and Omer et al., 2023) . Pomegranate peel extract reached 100% inhibition at the highest concentration (Figure 4), likely due to phenolics and tannins (Rani & Murty, 2006)

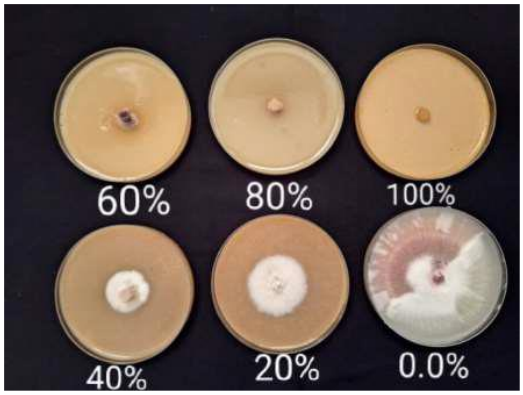

Figure 4. Effect of the cold aqueous extract of pomegranate on the growth of the fungus *F. oxysporum*

Eucalyptus leaf extract exhibited moderate antifungal activity, achieving approximately 56% inhibition at full-strength concentration (Figure 5). This response is consistent with earlier findings showing that extracts and essential oils from *Eucalyptus globulus*, *Eucalyptus citriodora*, and *Eucalyptus microcorys* possess measurable antifungal effects, particularly at higher concentrations. Musyimi and Ogur (2008) reported that eucalyptus essential oils produced clear inhibitory zones against dermatophytic fungi, with activity increasing markedly as concentration approached 100%. Similarly, Bhuyan et al. (2017) demonstrated that crude aqueous extracts of eucalyptus leaves suppressed the growth of several fungal species, attributing this effect to phenolic and flavonoid constituents. The moderate inhibition observed in the present study therefore aligns well with the established pattern described by these studies, indicating that eucalyptus leaf extract functions as a moderately effective antifungal agent whose activity becomes more evident at elevated concentrations.

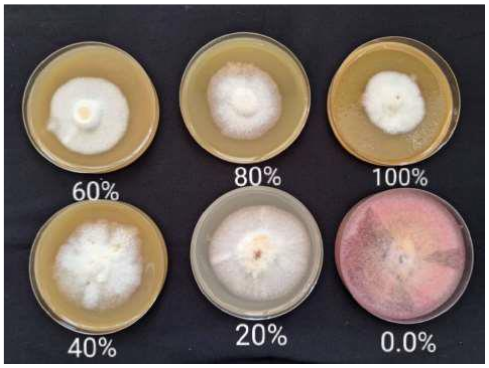

Figure 5. Effect of the cold aqueous extract of Eucalyptus on *F. oxysporum*

Rosemary and mint extracts showed weak antifungal activity in this study, with inhibition remaining below 35% (Figures 6 and 7). Such limited efficacy is consistent with reports indicating that both *Rosmarinus officinalis* and *Mentha piperita* generally exhibit modest antifungal effects when used as crude extracts. Freire et al. (2012) noted that peppermint essential oil produces only moderate inhibition at low concentrations, and similar findings were reported by Vakili-Ghartavol et al. (2022). Rosemary displays a comparable pattern; Kaab (2019) demonstrated that strong antifungal effects occur mainly with highly concentrated essential oils, whereas diluted or aqueous extracts show much lower activity. The low inhibition observed here therefore aligns well with the established literature, confirming that these plants act as mild antifungal agents under non-optimized extraction conditions.

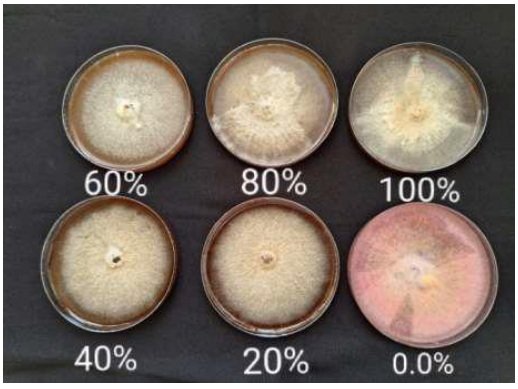

Figure 6. Effect of the cold aqueous extract of rosemary on the fungus *F. oxysporum*..

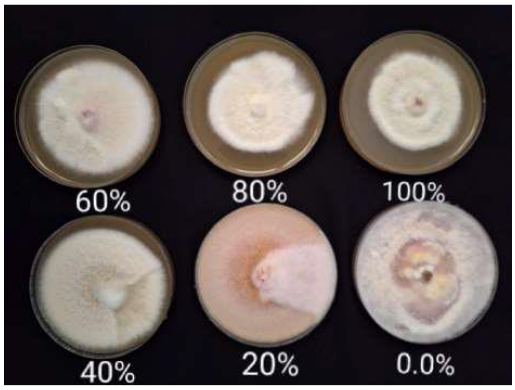

Figure 7. Effect of the cold aqueous extract of mint on the growth of *F. oxysporum* after two weeks of treatment.

Marjoram, wormwood, and oleander extracts showed minimal antifungal activity even at high concentrations (Figures 8, 9, and 10), which aligns with earlier findings by Ismail (2010) and Siddiqui et al. (2016). These studies reported that although these plants contain certain secondary metabolites, their crude aqueous extracts generally lack the potency needed to significantly inhibit fungal growth. Ismail (2010) noted that marjoram and wormwood exhibit only weak suppression of mycelial extension unless extracted with stronger organic solvents capable of isolating higher concentrations of active compounds. Similarly, Siddiqui et al. (2016) found that oleander's antifungal constituents are either present in low quantities or not effectively released through water-based extraction methods, resulting in limited inhibitory effects. The modest response observed in the current study therefore corresponds with the established literature, indicating that the antifungal potential of these plants may require alternative extraction techniques or higher-purity fractions to become fully effective.

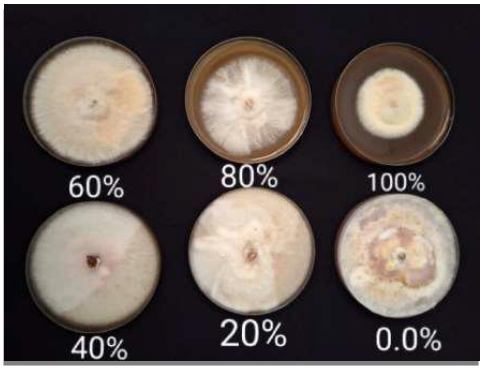

Figure 8. Effect of the cold aqueous extract of marjoram on *F. oxysporum*

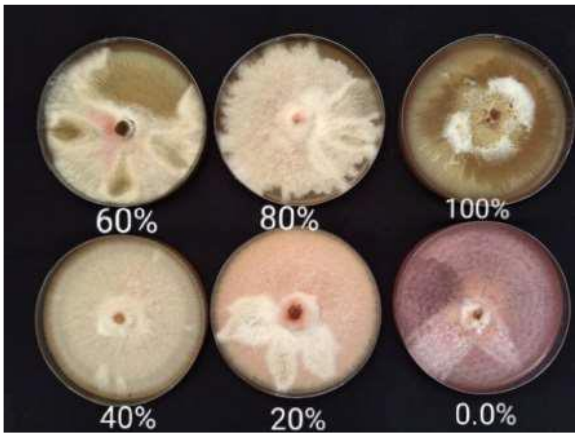

Figure 9. Effect of the cold aqueous extract of sagebrus on the growth of the fungus *F. oxysporum*

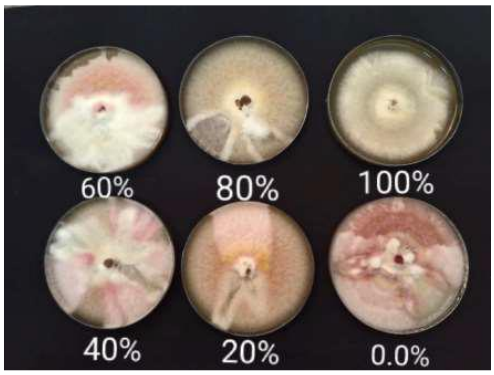

Figure 10. Effect of the cold aqueous extract of oleander on *F. oxysporum.*

Statistical analysis revealed significant differences in inhibition based on extract type and concentration. Clove extract recorded the highest average inhibition in all concentrations while oleander had the lowest as shown in Table 1.

Table 1. Effect of different concentrations of plant aqueous extracts on the percentage of growth inhibition of *F. oxysporumm* treatment

Inhibition *(I%)						Type of Plant
Conc%						
100	80	60	40	20	0	
100.00 a	100.00 a	100.00 a	100.00 a	92.22 b	0.00 x	Clover
100.00 a	100.00 a	100.00 a	86.92 c	60.00 fg	0.00 x	Thyme
100.00 a	90.29 b	83.33 d	70.29 e	57.59 gh	0.00 x	Pomegranate
100.00 a	100.00 a	60.00 f	53.89 j	48.88 k	0.00 x	Garlic
55.96 hj	48.89 k	41.48 m	28.61 op	25.92 qr	0.00 x	Eucalyptus
35.00 n	30.85 o	24.74 r	11.96 u	00.00 x	0.00 x	Mint
27.40 pq	23.89 r	20.55 s	17.38 t	12.96 u	0.00 x	Rosemary
41.48 m	25.18 qr	7.40 vw	1.66 x	00.00 x	0.00 x	Morjoram
16.92 t	8.88 v	6.96 vw	5.44 w	00.00 x	0.00 x	Wormwood
17.40 t	11.59 u	6.66 vw	0.00 x	00.00 x	0.00 x	Oleander

Effect on Spore Formation

Microscopic examination of fungal sporulation after 14 days showed clear variation in how the extracts affected conidial development. At concentrations of 40% and above, clove, thyme, and garlic extracts markedly inhibited the production of both macroconidia and microconidia (Figures 11–12-13).

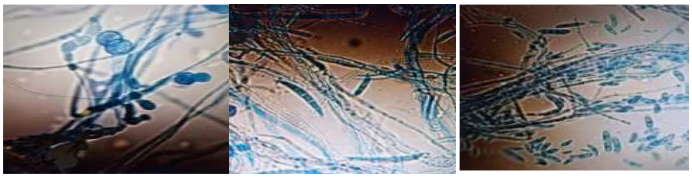

Figure 11. Types of spores produced by *F. oxysporum* after two weeks of treatment with the extract of clove.

Figure 12. Types of spores produced by *F. oxysporum* after treatment with the aqueous extract of thyme.

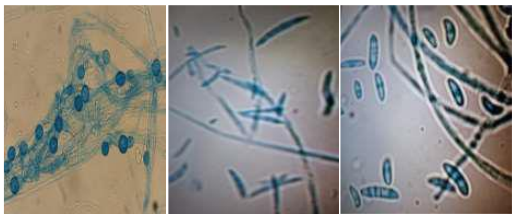

Figure 13. Types Aof spores produced by *F.oxysporum* after treatment with the aqueous extract of garlic.

Pomegranate and rosemary extracts also reduced sporulation at higher doses (Figures 14 and 15).

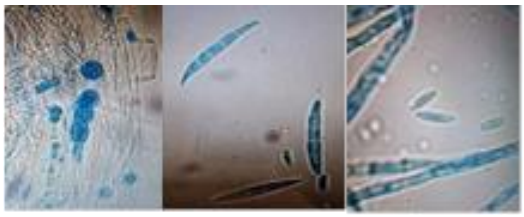

Figure 14. Types of spores produced by *F. oxysporum* after two weeks of treatment with the aqueous extract of pomegranate.

Figure 15. Types of spores produced by *F. oxysporum* after treatment with the aqueous extract of rosemary.

Chlamydospores, being thick-walled survival structures, were generally unaffected, a finding consistent with Elshafie & Camele (2021) and López-Meneses et al. (2022). Both studies emphasized that the resilience of chlamydospores is primarily due to their heavily reinforced cell walls and their ability to enter a metabolically inactive state, which allows them to withstand chemical, environmental, and biological stressors. Elshafie & Camele (2021) noted that many plant-derived antifungal compounds—including phenolics and volatile oils—readily inhibit hyphal growth and conidial formation but rarely penetrate or disrupt chlamydospore walls. Similarly, López-Meneses et al. (2022) demonstrated that chlamydospores of Fusarium spp. maintain structural integrity even under exposure to potent bioactive extracts, enabling the fungus to survive adverse conditions and later re-colonize host tissues. The limited impact observed in the present study therefore reflects the inherent durability of these structures and supports the conclusion that suppressing chlamydospore viability may require more targeted or intensive strategies beyond aqueous plant extracts.

Variations in spore suppression are attributed to differences in phytochemical content—particularly phenolics, flavonoids, and essential oils (Samy et al., 2023; Sharma et al., 2021). According to Samy et al. (2023), these bioactive compounds act through multiple mechanisms, including disrupting cell wall integrity, altering membrane permeability, and inhibiting key enzymes required for conidial development. Sharma et al. (2021) further explained that extracts rich in phenolic and flavonoid compounds interfere with fungal metabolic pathways and oxidative balance, leading to reduced spore viability and impaired germination. Essential oils, in particular, exert strong lipophilic interactions with fungal membranes, causing leakage of intracellular contents and inhibiting sporulation. Therefore, the observed differences among plant extracts in this study can be directly linked to the qualitative and quantitative variation in their phytochemical profiles, which determines how effectively each extract suppresses macroconidia and microconidia formation. **Phytochemical Composition and Correlation with Activity**

Phytochemical screening results are presented in Table 2, showing that clove, pomegranate, and marjoram contained a broad spectrum of bioactive metabolites,

including flavonoids, alkaloids, and terpenoids, which are widely recognized for their strong antifungal properties. The richness and diversity of these secondary compounds help explain the pronounced inhibitory effects observed against F. oxysporum, as each group of metabolites contributes differently to disrupting fungal membranes, inhibiting enzyme systems, and suppressing sporulation. Garlic, while lacking saponins and glycosides, contained allicin—a highly reactive sulfur-based compound known for its potent antimicrobial and antifungal action—supporting earlier findings by Kutawa et al. (2018) regarding its ability to impair fungal growth through oxidative and membrane-disruptive mechanisms. In contrast, wormwood and oleander extracts exhibited limited phytochemical diversity, containing fewer active constituents capable of exerting strong antifungal effects. This scarcity of key metabolites aligns with the weak inhibitory activity recorded in this study and is consistent with the observations of Bokhari et al. (2013), who similarly reported poor antifungal performance of these plants when extracted with aqueous solvents. Together, these results highlight the strong correlation between phytochemical richness and antifungal potency.

Table 2. Preliminary chemical screening results of active components in plant aqueous extracts

Active chemical compounds								Type of Plant
Phenols	Flavones	Alkaloids	Glycosides	Terpenes	Saponins	Resins	Tannins	
+	+	+	+	+	+	+	+	Clover
+	+	+	+	+	+	+	+	Thyme
+	+	+	-	+	-	-	+	Garlic
+	+	+	+	+	+	+	+	Pomegranate
+	-	-	+	-	+	-	+	Blue gume

Active chemical compounds								Type of Plant
Phenols	Flavones	Alkaloids	Glycosides	Terpenes	Saponins	Resins	Tannins	
+	-	+	+	+	+	+	+	Mint
+	+	+	+	+	+	+	+	Mojoram
+	+	+	+	+	+	-	+	Rosemary
-	-	-	+	+	+	-	-	Oleander
+	+	+	+	+	+	-	+	Wormwood
(+) Evidence of the presence of the chemical compound. (-)Evidence of the absence of the chemical compound.								

Physicochemical analysis of the extracts (Table 3) showed pH values ranging from 3.6 (pomegranate) to 6.3 (rosemary), which may influence extract stability and fungal response.**Table 3. Some physical properties of the plant aqueous extracts.**

Acidity (pH)	Color	Plant Extract (Used Part)
4.2	Dark brown	Clove – Flower buds
6	Light green	Thyme – Leaves
5.5	Light yellow	Garlic – Cloves
3.6	Reddish brown	Pomegranate – Peels
5.1	Light brown	Cypress – Leaves
6	Green	Mint – Leaves
5.6	Dark green	Marjoram – Leaves
6.3	Green	Rosemary – Leaves
5.9	Yellowish green	Oleander – Leaves
6.1	Light green	Wormwood – Leaves

Overall, the presence of bioactive compounds such as flavonoids, tannins, phenolics, and volatile oils was positively associated with antifungal efficacy (Cushnie & Lamb, 2005; Cowan, 1999). According to Cushnie & Lamb (2005), flavonoids and phenolic compounds exert their antifungal action primarily through destabilizing cell membranes, chelating essential metal ions, and generating oxidative stress within fungal cells, ultimately impairing growth and reproduction. Cowan (1999) further highlighted that tannins interfere with fungal enzymes by forming irreversible complexes with proteins, while volatile oils penetrate lipid layers, increasing membrane permeability and causing leakage of cellular contents. These mechanisms collectively help explain the strong inhibitory effects observed in extracts rich in these constituents, as they not only suppress mycelial growth but also hinder the processes involved in conidial formation and germination. Thus, the phytochemical profile of each extract plays a decisive role in determining its antifungal potential

Conclusion

The findings of this study clearly demonstrate that cold aqueous extracts from several locally available plant species in Libya possess significant antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of tomato wilt disease. Among the tested extracts, clove, garlic, thyme, and pomegranate peel proved to be the most effective, achieving complete inhibition of mycelial growth and conidial formation at concentrations of 40% and above. This strong activity is attributed to the abundance of bioactive secondary metabolites—such as phenolics, flavonoids, terpenoids, tannins, and alkaloids—which are known to disrupt fungal cell structures and interfere with essential metabolic pathways.

In contrast, extracts from plants such as wormwood and oleander exhibited limited antifungal potential, underscoring the direct relationship between phytochemical diversity and inhibitory capacity. Microscopic observations further confirmed that the most potent extracts significantly reduced the formation of macroconidia and microconidia, while chlamydospores were less affected, highlighting differences in fungal resilience and structural resistance.

Collectively, these results provide strong evidence that aqueous plant extracts—particularly those derived from clove, garlic, thyme, and pomegranate—offer promising, eco-friendly alternatives to synthetic fungicides. Their effectiveness, affordability, and local availability make them viable candidates for integration into sustainable disease management strategies. Future

studies should extend these findings through greenhouse and field evaluations, explore formulation improvements, and assess their compatibility within integrated pest management programs. Advancing such plant-based solutions represents an important step toward achieving safer, more sustainable tomato production systems in Libya.

Based on the findings of this study, it is recommended that the most effective plant extracts—particularly those derived from clove, garlic, thyme, and pomegranate—be further evaluated under greenhouse and field conditions to verify their practical reliability in managing *Fusarium oxysporum* wilt in tomato crops. Future research should also focus on developing standardized biofungicidal formulations from these extracts, optimizing extraction techniques to enhance the concentration of active compounds, and conducting advanced phytochemical profiling using analytical tools such as GC–MS and HPLC to identify the key metabolites responsible for antifungal activity. Additional studies exploring the exact modes of action of these extracts, together with safety and toxicity assessments, are essential to ensure their compatibility with plants, beneficial soil microorganisms, and human health. Integrating these promising botanical extracts into sustainable Integrated Pest Management (IPM) programs and expanding their evaluation to other fungal pathogens and economically important crops will further support the development of environmentally friendly alternatives to conventional chemical fungicides in Libya.

Ethical Considerations

All experimental procedures in this study were conducted in accordance with standard laboratory safety and ethical guidelines for handling plant materials and phytopathogenic fungi. The collection of plant samples was limited to non-endangered species and performed without causing harm to natural habitats. Isolation and culturing of *Fusarium oxysporum* were carried out under aseptic conditions to prevent environmental contamination and ensure researcher safety. No experiments involved humans or vertebrate animals; therefore, no institutional ethical approval was required. All data were generated honestly, without fabrication or manipulation, and the research adhered to principles of scientific integrity and responsible conduct.

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