



# Biological Study on *Alternaria alternata* isolated from infected Vegetables in Sana'a, Yemen

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## ABSTRACT

*Alternaria alternata*, the causal agent of early blight, is one of the most destructive pathogens affecting tomato and potato crops in Yemen, with documented yield losses of up to 80%. The traditional reliance on synthetic fungicides has led to environmental degradation and the emergence of resistant strains, necessitating the development of sustainable, eco-friendly alternatives. This study aimed to isolate and identify *A. alternata* from infected crops in the Sana'a Governorate and evaluate the in vitro antifungal efficacy of essential oils (EOs) derived from *Cupressus macrocarpa* leaves. Of the 41 initial isolates, 19 were identified as *A. alternata* based on morphological and microscopic characterization. Pathogenicity assays revealed significant variability in virulence, with isolate 1T1 (from tomato) exhibiting the highest disease severity (56%). Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified the major chemical constituents, including monoterpenes (4-terpineol,  $\gamma$ -terpinene, and  $\alpha$ -terpinene) and fatty acid esters. In vitro assays demonstrated potent fungicidal activity; at concentrations of 0.2%, 0.4%, and 0.6%, the EO achieved up to 97% mycelial growth inhibition at the highest concentration. This efficacy was comparable to that of the synthetic fungicide difenoconazole (0.1%). These findings suggest that *C. macrocarpa* essential oil is a promising biofungicide candidate for sustainable management of Alternaria blight.

## ARTICLE INFO

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*Alternaria alternata*, Early blight, potato and tomato diseases, *Cupressus macrocarpa*, Essential oils, Antifungal Activity, biofungicide.

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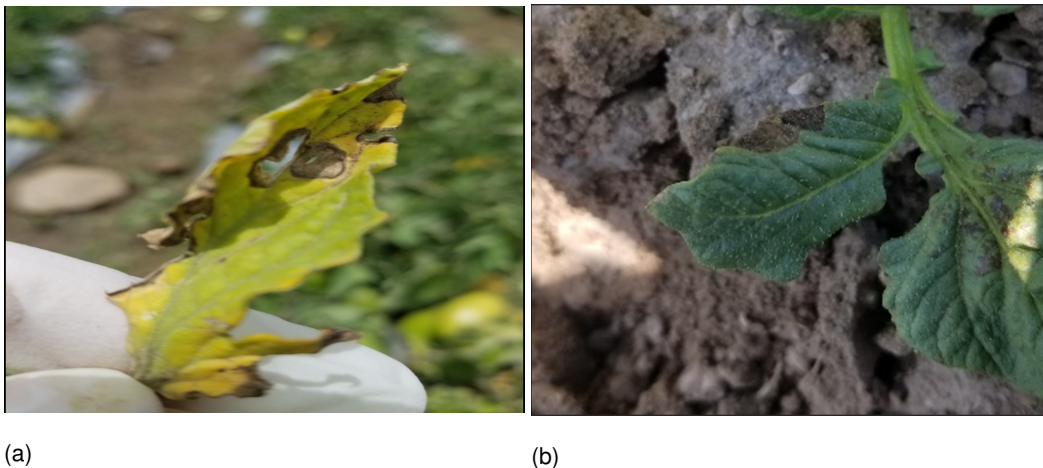
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## 1. INTRODUCTION

The genus *Alternaria* was first introduced by [1]. Currently, the genus *Alternaria* belongs to the family *Pleosporaceae* (Pleosporales, Dothideomycetes, Ascomycota) [2]. The *Alternaria* genus has a wide variety of species around the world. Approximately 400 plant species are hosts of *Alternaria* species, including various species of crops, fruits, vegetables, ornamentals, and weeds. Specifically, species such as *Alternaria solani* and *Alternaria alternata* are the primary causal agents of early blight disease in the *Solanaceae* family crops, including potato, tomato, pepper, and eggplant [3–5]. *A. alternata* is an endophytic species and is among the important seed-borne plant pathogens. *A. alternata* can produce more than 30 mycotoxins, and these toxins cause tissue necrosis with enzymes that degrade the cell wall

[6, 7]. Agricultural equipment and infected seeds. This pathogen's spores can enter the plant's leaves, stems, and fruits. Early blight caused by *A. solani* Sorauer and *A. alternata* is an economically important and widely distributed disease worldwide on crops belonging to the *Solanaceae* family [8]. The disease occurs on leaves, stems, petioles, and fruits in high-humidity and high-temperature areas. The pathogen prefers mature tissues and is more frequent during the fruiting period, causing high economic losses [9, 10]. Early blight causes 50% to 80% yield loss, especially in tomato production [11].

*Alternaria* species have a wide host range, extreme variability, and long and active vegetative phases, and are very difficult to control [12]. Various methods, such as crop rotation, resistant varieties, soil fumigation, and fungicide application, can be used to control the disease [13, 14]. Synthetic fungicides used as both seed and



**Figure 1.** Infected plant samples (a), tomato (b), potato.

spray treatments include Captan, Ridomil, Strobilurin, Iprodione, Mancozeb, Carbendazim, and Difenconazole [15–18]. However, synthetic fungicides accumulate in the soil, animals, and plants, adversely affecting ecosystems. Additionally, fungal agents become resistant to fungicides over time, reducing their effectiveness [19]. In this context, it has become necessary to research and develop alternative methods to chemical controls that are friendly to human health and the environment for controlling fungal diseases [20].

Essential oils, also known as ethereal oils, are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots) and do not contain synthetic chemicals. They stand out as natural fungicides for controlling plant pathogens [21, 22]. Essential oils containing active compounds such as sesquiterpenes, monoterpenes, and oxygenated compounds exhibit antimicrobial activity [23]. These compounds cause the separation of lipid layers from the fungal cell membrane, disruption of cell membrane integrity and permeability by altering membrane structure, and metabolic deterioration in cytoplasmic and mitochondrial structures [24–26]. The ‘ ‘ Cypress plants belong to the family *Cupressaceae* and are grown in many subtropical areas for commercial purposes, such as ornamentation, and as a source of wood-building material. *Cupressus macrocarpa* (Hartweg. Ex. Gordon), as a medicinal plant, belongs to this family and is commonly known as Monterey Cypress. Several *Cupressus* species have been investigated for their essential oil content and evaluated for biological activity. The study conducted in Egypt identified 43 components from the fresh and dried leaves of the Egyptian *C. macrocarpa* with significant antibacterial and antifungal activities [27]. This study aimed to isolate and identify the *Alternaria* fungus responsible for early blight disease in *potato* and *tomato* crops in cultivated regions of Yemen and evaluate the antifungal efficacy of *Cupressus macrocarpa* essential oil against the isolated *Alternaria* fungus un-

der laboratory conditions. To the best of our knowledge, this is the first report to assess the antifungal efficacy of lemon cypress (*C. macrocarpa*) essential oil as a safe organic alternative against *A. alternata* in Yemen.

## 2. MATERIALS AND METHODS

This study was performed at the microbiology laboratory of the Agriculture Veterinary Technical Institute in Sana’a, Yemen, from 2024 to 2025.

### 2.1. SAMPLE COLLECTION AND PATHOGEN ISOLATION

Samples of *Solanaceae* plants (tomato and potato) showing typical symptoms of early blight were collected from infected fields in the Sana’a governorate (Fig. 1).

For isolation, small pieces of infected tissues were surface-sterilized with a 1% sodium hypochlorite solution, cultured on potato dextrose agar (PDA), and incubated at  $25 \pm 1^\circ\text{C}$  [28, 29]. The isolates (19 of 41 isolates) were identified based on morphological and microscopic characteristics, and conidia measurements were performed using an ocular micrometer [30–34].

### 2.2. PATHOGEN IDENTIFICATION, CULTURAL AND MORPHOLOGICAL STUDIES

The 19 isolates were identified based on their morphological characteristics at the microbiology laboratory of the Agriculture and Veterinary Technical Institute, Sana’a. For each isolate, three 5-mm-diameter plugs were transferred onto three plates containing PDA for *A. alternata* and incubated at  $25 \pm 1^\circ\text{C}$  under 10/14 h fluorescent light/darkness for 7 days. Cultures were examined for growth rate, colony color, colony margin, colony texture, and the development of pigments or crystals in the agar medium [30–33]. Sporulation of all *A. alternata* isolates was performed as described by [35]. A microscopic de-

scription of the conidia was obtained using slide culture techniques [36]. A total of 100 conidia were used for measurements of length, width (all the measurements were done with a light microscope at x40 objective lens with the help of ocular micrometer), and septa numbers (per conidia) [37].

### 2.3. PATHOGENICITY TESTS

Pathogenicity tests were conducted under greenhouse conditions using commercial varieties of tomato (Vilmorin-Sphera F1) and potato (Diamant). Plants were grown in a sterilized soil mixture (clay loam: sand: peat moss in a 3:1:1 ratio) [38, 39]. Fungal inoculum was prepared from 10-day-old *A. alternata* cultures, and the spore suspension was adjusted to  $5 \times 10^4$  conidia/ml.

Plants were inoculated by spraying at the 4-6 true leaves stage (three replicates), with sterile distilled water used as a negative control. The pathogen was re-isolated to complete Koch's postulates [34]. Disease severity was measured on a scale of 0–5: (1) level 0: no symptoms, resistant; (2) level 1: 1%–5% of spots on the leaves, moderately resistant; (3) level 2: 6%–20% of spots on the leaf, moderately resistant; (4) level 3: 21%–40% of spots on the leaves, moderately susceptible; (5) level 4: 41%–60% of spots on the leaves, moderately susceptible; and (6) level 5: more than 61% of spots on the leaves, susceptible [10]. Disease severity has been used to determine a disease severity index (DSI) on a percentage basis, where

$$\text{DSI \%} = \frac{\text{Sum of individual rating}}{(\text{No. of leaves sampled} \times \text{maximum disease scale})} \times 100 \quad (1)$$

### 2.4. EXTRACTION AND CHEMICAL ANALYSIS OF VOLATILE OIL

The aerial parts of *Cupressus macrocarpa* (Fig. 2) were collected from Sana'a between 2024 and 2025 and identified by botanists (Dr. Abdallah Amen) at the Department of Plant Protection, Faculty of Agriculture and Environment, Sana'a University.



Figure 2. Plant sample *Cupressus macrocarpa*.

#### 2.4.1. Extraction:

Essential oils were extracted from 100 g of leaves of the plant using a Clevenger-type apparatus via hydrodistillation for three hours. The extracted oils were dried over

anhydrous sodium sulfate and stored in a refrigerator at 4°C [40].

#### 2.4.2. Chemical Analysis:

The pure essential oil was diluted in absolute ethanol (99.9%) to a final concentration of 0.001% (v/v) and subsequently analyzed by gas chromatography-mass spectrometry (GC-MS) (Shimadzu QP2020 NX GC with Shimadzu 2030 MSD) at the Yemen Standardization Metrology and Quality Control Organization, Sana'a. The components were identified based on their retention indices and by comparing the mass spectral fragmentation patterns with those in the NIST database [41].

### 2.5. EVALUATION OF ESSENTIAL OILS' EFFICACY IN VITRO

The antifungal activity of the essential oils against *Alternaria alternata* was evaluated using the Poisoned Food Technique at three concentrations (2, 4, and 6  $\mu\text{L/mL}$ ), corresponding to volume ratios of 0.2%, 0.4% and 0.6% in the substrate [42]. Pure PDA was used as the negative control. The commercial fungicide, Difenoconazole 25 EC (0.1%), was used as a positive control. Five mm culture discs from one-week-old cultures of *A. alternata* were cut using a sterile cork borer and cultured at the center of each petri dish per replicate and incubated for 7 days at  $27 \pm 1^\circ\text{C}$ . Fungal growth was measured for each treatment on the 1st, 3rd, 5th, and 7th days. The Minimum Inhibitory Concentration (MIC) was the minimum dosage that resulted in complete mycelial inhibition. All MIC assessments were conducted in triplicate ( $n = 3$ ) to ensure repeatability. The percentage of inhibition of mycelial growth was calculated using the formula given by [43].

$$I = \frac{C - T}{C} \times 100 \quad (2)$$

where I = percentage inhibition of fungal growth. C = Growth of fungal colony diameter (mm) in the control. T = Growth of fungal colony diameter (mm) in treatment.

The mean radial growth of the test pathogen on the amended media was compared with that of the negative and positive controls [44, 45].

## 2.6. DATA ANALYSIS

Data on the morphological characterization of *A. alternata* were analyzed through colony and microscopic examination. The chemical composition of the essential oils was analyzed using an auto sampler gas chromatography-mass spectrometry (GC-MS) system. All measured data were statistically analyzed using Stata Statistical Software (STATA).

## 3. RESULT AND DISCUSSION

### 3.1. ISOLATION AND IDENTIFICATION OF THE CAUSAL ORGANISM

From August to October 2024, naturally infected samples of tomato and potato showing typical early blight symptoms were collected from different locations (Hamdan, Bani Aseem, and Al Rahab) in the Sana'a governorate. A total of 30 isolates from tomato samples and 11 isolates from potato samples were obtained.

The causal organism was isolated from infected tissues using the tissue isolation method on a sterilized PDA medium. The cultures were purified and maintained on PDA slants through periodic transfers. Microscopic examination confirmed the presence of *A. alternata* in the diseased tissue. Based on its cultural and morphological characteristics, the isolated pathogen was identified as *A. alternata*.

Isolation of *A. alternata* from tomato on PDA media using the tissue isolation method was also demonstrated by [46, 47]. Also, isolation of *A. alternata* from potatoes was also reported by Madadi, et al. [48], which coincides with the results of the present study.

### 3.2. PATHOGEN CULTURAL AND MORPHOLOGICAL STUDIES

Isolates of *A. alternata* from tomato and potato exhibited considerable variability on the PDA medium. The colony color ranged from gray to olive cottony with profuse aerial mycelium in young cultures, gradually turning dark gray to olive-black upon sporulation. Mature colonies were entirely black with 3–4 concentric rings, dense growth, and distinct white margins. Growth was relatively slow, reaching 90 mm in diameter after 14 days at  $28 \pm 1$  °C (Fig. 3A).

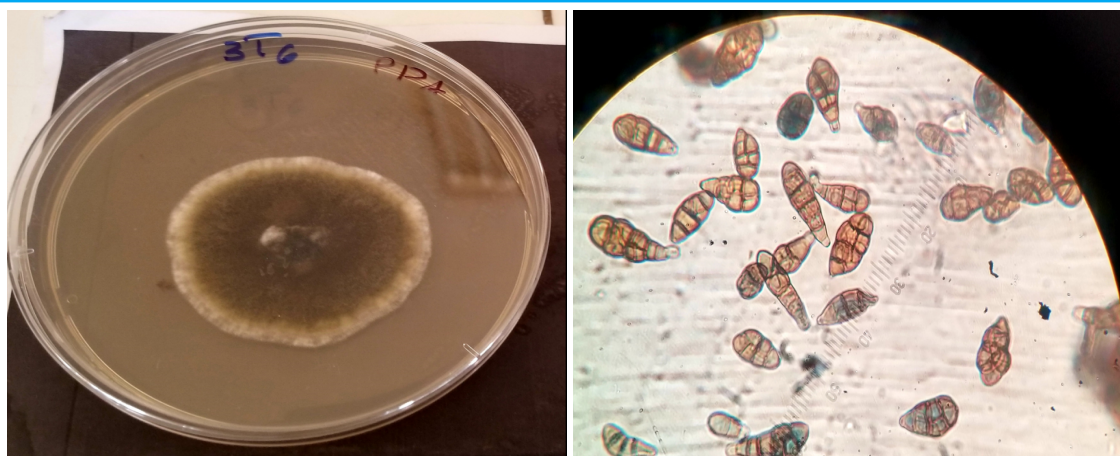
Microscopic identification of the isolates confirmed the typical morphology of *A. alternata* (Fig. 3B).

On tomato (Table 1), conidiophores were solitary, straight or curved, measuring  $18.2\text{--}59.6 \mu\text{m} \times 3.3\text{--}7.2 \mu\text{m}$ . Conidia formed in branching chains, ovoid to ellipsoidal, brown. conidial measurements and septation. The mean conidial length across all isolates was  $29.7 \pm 9.4 \mu\text{m}$ . Isolate 1T1 (Al Rahab) exhibited the longest conidia, averaging  $39.5 \pm 10.4 \mu\text{m}$  (range:  $27.5\text{--}52.5 \mu\text{m}$ ), while isolates 1T2 and 1T3 had the shortest, with mean lengths of  $22.5 \pm 5.9 \mu\text{m}$  and  $22 \pm 2.9 \mu\text{m}$ , respectively. Conidial width averaged  $10.9 \pm 2.6 \mu\text{m}$ , with isolates 1T1 and 3T6 showing the greatest widths ( $13 \pm 2.7 \mu\text{m}$  and  $13 \pm 1.1 \mu\text{m}$ , respectively), and isolate 1T5 presenting the narrowest ( $9 \pm 1.4 \mu\text{m}$ ). The number of septa per conidium ranged from 2 to 7, with an overall mean of  $4.3 \pm 1.5$ . Isolate 1T1 also displayed the highest mean septa count ( $6.2 \pm 0.8$ ), whereas isolates 1T2, 3T3, and 3T5 had the lowest ( $3 \pm 0.7$ ). Isolates from Bani Aseem generally demonstrated broader morphological diversity, including conidial lengths from  $17.5 \mu\text{m}$  to  $47.5 \mu\text{m}$  and 2 to 7 septa. Statistical analysis confirmed significant differences in conidial length, width, and septa number among the isolates, indicating considerable morphological diversity within the *A. alternata* population in the studied regions.

On potato (Table 2), conidiophores were similar in form and size, while the measurements of conidia and septation were distinct. The overall mean conidial length was  $25.9 \pm 11.0 \mu\text{m}$ . Isolate 3P1 from Hamddan exhibited the largest conidia, with a mean length of  $43 \pm 5.1 \mu\text{m}$  (range:  $37.5\text{--}47.5 \mu\text{m}$ ). Conversely, isolates from Bani Mater (4P2, 4P3), Al Rahab (2P), Pb1, and Pb3 exhibited significantly shorter conidia, with mean lengths ranging from  $18.5 \pm 1.4$  to  $20.5 \pm 3.3 \mu\text{m}$ . Conidial width averaged  $10.1 \pm 2.9 \mu\text{m}$  across all samples, with isolate 3P1 also possessing the widest conidia ( $14.5 \pm 3.3 \mu\text{m}$ ), while isolate Pb3 had the narrowest ( $8 \pm 1.1 \mu\text{m}$ ). The total mean number of septa per conidium was  $3.5 \pm 1.6$ . Isolate 3P1 was distinct with the highest septa count ( $6.4 \pm 1.1$ ). In contrast, the remaining six isolates consistently exhibited reduced septation, varying from 2 to 4, with mean values between 2.6 and 2.8. These findings indicate that isolate 3P1 from Hamddan is morphologically distinct, characterized by significantly larger and more septate conidia, while the remaining isolates form a more homogenous group. A similar type of cultural and morphological characters was also observed by [36, 46, 48–50].

### 3.3. PATHOGENICITY TEST

Eight isolates of *A. alternata* (six from tomato and two from potato) were tested for pathogenicity. Lesion areas were measured at 20 days post-inoculation (DPI). Early blight caused by *A. alternata* initially produced



(A) (B)

Figure 3. Showing (A) cultural and (B) microscopic identification of *A. alternata* isolates.

Table 1. Morphological comparisons among twelve *A. alternata* isolates on the tomato crop from two different locations

| Isolates | Location   | Conidia size ( $\mu\text{m}$ ) |                                |                |   | Septa No.     |                                  |
|----------|------------|--------------------------------|--------------------------------|----------------|---|---------------|----------------------------------|
|          |            | Length                         |                                | Width          |   | Range         | Mean*                            |
|          |            | Range                          | Mean*                          | Range          | Mean*                                     |               |                                  |
| 1T1      | Al Rahabah | 27.5-52.5                      | 39.5 $\pm$ 10.4 <sup>cdg</sup> | 10-12.5        | 13 $\pm$ 2.7 <sup>d g</sup>               | 5-7           | 6.2 $\pm$ 0.8 <sup>b e</sup>     |
| 1T2      |            | 12.5-27.5                      | 22.5 $\pm$ 5.9 <sup>a</sup>    | 7.5-12.5       | 11 $\pm$ 2.2 <sup>abcde</sup>             | 2-4           | 3 $\pm$ 0.7 <sup>a</sup>         |
| 1T3      |            | 17.5-25                        | 22 $\pm$ 2.9 <sup>a</sup>      | 7.5-12.5       | 9.5 $\pm$ 2.1 <sup>abcdef</sup>           | 3-4           | 3.4 $\pm$ 0.5 <sup>a c</sup>     |
| 1T4      |            | 17.5-35                        | 24 $\pm$ 2.2 <sup>a</sup>      | 10-15          | 12 $\pm$ 2.9 <sup>d g</sup>               | 2-6           | 3.4 $\pm$ 1.5 <sup>a c</sup>     |
| 1T5      |            | 15-47.5                        | 25 $\pm$ 8.9 <sup>ae h</sup>   | 7.5-12.5       | 9 $\pm$ 1.4 <sup>abcdef</sup>             | 2-6           | 3.4 $\pm$ 1.5 <sup>a c</sup>     |
| 3T1      | Bani Aseem | 25-47.5                        | 34 $\pm$ 9.4 <sup>bcd f</sup>  | 10-12.5        | 11.5 $\pm$ 1.4 <sup>abcd g</sup>          | 4-6           | 4.2 $\pm$ 1.1 <sup>a c d f</sup> |
| 3T2      |            | 27.0-47.5                      | 30.5 $\pm$ 8.0 <sup>bcdg</sup> | 7.5-17.5       | 9.5 $\pm$ 2.1 <sup>abcdef</sup>           | 4-6           | 4.8 $\pm$ 1.3 <sup>bcd f</sup>   |
| 3T3      |            | 20-32.5                        | 26 $\pm$ 5.2 <sup>ae f h</sup> | 10-17.5        | 12.5 $\pm$ 3.1 <sup>b<sup>d</sup> g</sup> | 2-4           | 3 $\pm$ 0.7 <sup>a</sup>         |
| 3T4      |            | 22.5-37.5                      | 31 $\pm$ 6.8 <sup>be f h</sup> | 7.5-12.5       | 10.5 $\pm$ 2.1 <sup>abcdef</sup>          | 3-6           | 4.8 $\pm$ 1.3 <sup>bcd f</sup>   |
| 3T5      |            | 17.5-27.5                      | 22 $\pm$ 2.2 <sup>a</sup>      | 7.5-12.5       | 10 $\pm$ 1.4 <sup>abcdef</sup>            | 2-5           | 3 $\pm$ 1.1 <sup>a</sup>         |
| 3T6      |            | 37.5-40                        | 38 $\pm$ 1.4 <sup>bcdg</sup>   | 12.5-15        | 13 $\pm$ 1.1 <sup>d g</sup>               | 5-7           | 6 $\pm$ 1.0 <sup>b e</sup>       |
| 3T7      |            | 17.5-32.5                      | 23 $\pm$ 7.4 <sup>a</sup>      | 7.5-15         | 9 $\pm$ 3.4 <sup>abc ef</sup>             | 2-6           | 3.8 $\pm$ 1.5 <sup>a cd</sup>    |
| Mean     |            | 29.7 $\pm$ 9.4                 |                                | 10.9 $\pm$ 2.6 |   | 4.3 $\pm$ 1.5 |                                  |

\*Means of length, width, and septa are presented as mean  $\pm$  standard deviation. Statistical comparisons were performed within each column using Duncan's Multiple Range Test (DMRT) at  $p \leq 0.05$ . Means followed by different letters are significantly different ( $p \leq 0.05$ ), whereas those followed by the same letter are not significantly different.

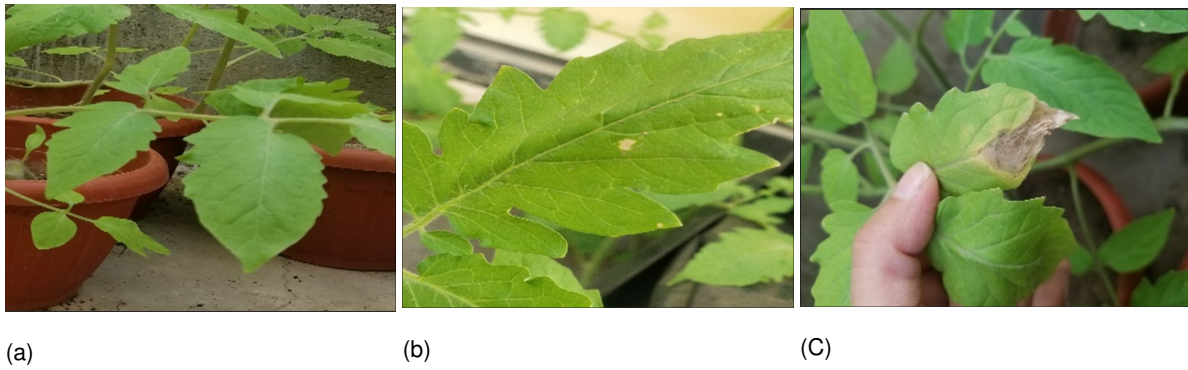
Table 2. Morphological comparisons among seven *A. alternata* isolates on potato crop from three different locations

| Isolates   | Location   | Conidia size ( $\mu\text{m}$ ) |                             |                |                                  | SeptaNo.      |                                |
|------------|------------|--------------------------------|-----------------------------|----------------|----------------------------------|---------------|--------------------------------|
|            |            | Length                         |                             | Width          |                                  | Range         | Mean*                          |
|            |            | Range                          | Mean*                       | Range          | Mean*                            |               |                                |
| 2P         | Al Rahabah | 17.5-25                        | 20.5 $\pm$ 3.3 <sup>a</sup> | 7.5-12.5       | 9.5 $\pm$ 2.1 <sup>abcdef</sup>  | 2-4           | 2.8 $\pm$ 0.8 <sup>a</sup>     |
| 3P1        | Hamddan    | 37.5-47.5                      | 43 $\pm$ 5.1 <sup>g</sup>   | 12.5-20        | 14.5 $\pm$ 3.3 <sup>abcdef</sup> | 5-8           | 6.4 $\pm$ 1.1 <sup>e</sup>     |
| Pa1        |            | 25-50                          | 40 $\pm$ 9.5 <sup>dg</sup>  | 10-12.5        | 10.5 $\pm$ 1.1 <sup>abcdef</sup> | 4-6           | 4.8 $\pm$ 0.8 <sup>bcd f</sup> |
| Pb1        |            | 17.5-30                        | 21.5 $\pm$ 5.8 <sup>a</sup> | 7.5-12.5       | 9.5 $\pm$ 2.7 <sup>abcdef</sup>  | 2-4           | 2.8 $\pm$ 0.8 <sup>a</sup>     |
| Pb3        |            | 17.5-25                        | 19 $\pm$ 2.2 <sup>a</sup>   | 7.5-12.5       | 8 $\pm$ 1.1 <sup>c ef</sup>      | 2-4           | 2.6 $\pm$ 0.9 <sup>a</sup>     |
| 4P2        | Bani Mater | 17.5-25                        | 18.8 $\pm$ 1.3 <sup>a</sup> | 10-12.5        | 9.5 $\pm$ 2.7 <sup>abcdef</sup>  | 2-4           | 2.8 $\pm$ 0.8 <sup>a</sup>     |
| 4P3        |            | 17.5-25                        | 18.5 $\pm$ 1.4 <sup>a</sup> | 10-12.5        | 9.5 $\pm$ 2.7 <sup>abcdef</sup>  | 2-4           | 2.6 $\pm$ 0.9 <sup>a</sup>     |
| Total Mean |            | 25.9 $\pm$ 11.0                |                             | 10.1 $\pm$ 2.9 |                                  | 3.5 $\pm$ 1.6 |                                |

\*Mean of the length, width, and septa followed by  $\pm$  standard deviation. Statistical comparisons were made within a single column using Duncan's Multiple Range Test (DMRT) at  $P \leq 0.05$ . Means followed by different letters are significantly different ( $P = 0.05$ ), whereas means followed by the same letter are not significantly different.

small, necrotic, scattered, brown, circular spots with varying sizes extending up to one centimeter. These spots grew larger and became irregularly shaped, forming big patches on the leaves with a blighted appearance, char-

acterized by typical concentric rings (Figs. 4 and 5). The disease symptoms match the observations made by various workers as reported by [46, 51, 52]. Significant differences in disease severity were observed among iso-



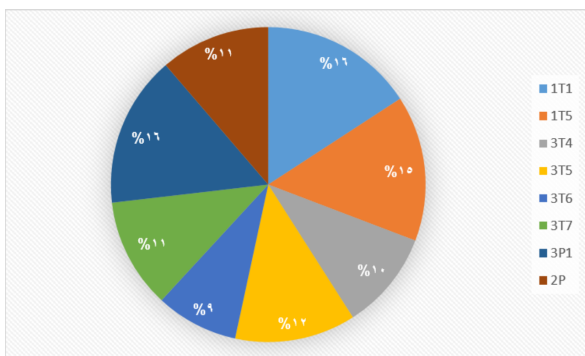
**Figure 4.** Symptoms of early blight on tomato leaves (a. healthy leaf. b. early infection. C. advanced infection)



**Figure 5.** Symptoms of early blight on potato leaves (a. healthy leaf. b. early infection. c. advanced infection)

lates. On tomato, isolate 1T1 caused the highest severity ( $56 \pm 1.732\%$ ), followed by 1T5 ( $53 \pm 1.732\%$ ) and 3T5 ( $44 \pm 1.732\%$ ), while isolates 3T7 ( $40 \pm 1.000\%$ ) and 3T6 ( $30 \pm 2.000\%$ ) showed the lowest severity. On potato, isolate 3P1 ( $55 \pm 1.732$ ) produced the highest severity, whereas isolate 2P ( $40 \pm 2.000$ ) showed the lowest. Overall, disease severity was significantly greater at 20 DPI compared to earlier evaluations (Fig. 6).

Similarly, pathogenicity tests in tomato and potato through artificial inoculation with spray suspension were also confirmed in *A. alternata*, as studied by [38, 46, 49], who used a spore suspension of  $10^4$  conidia/mL. They found that the different *Alternaria alternata* isolates differed in their pathogenicity (virulent and avirulent).

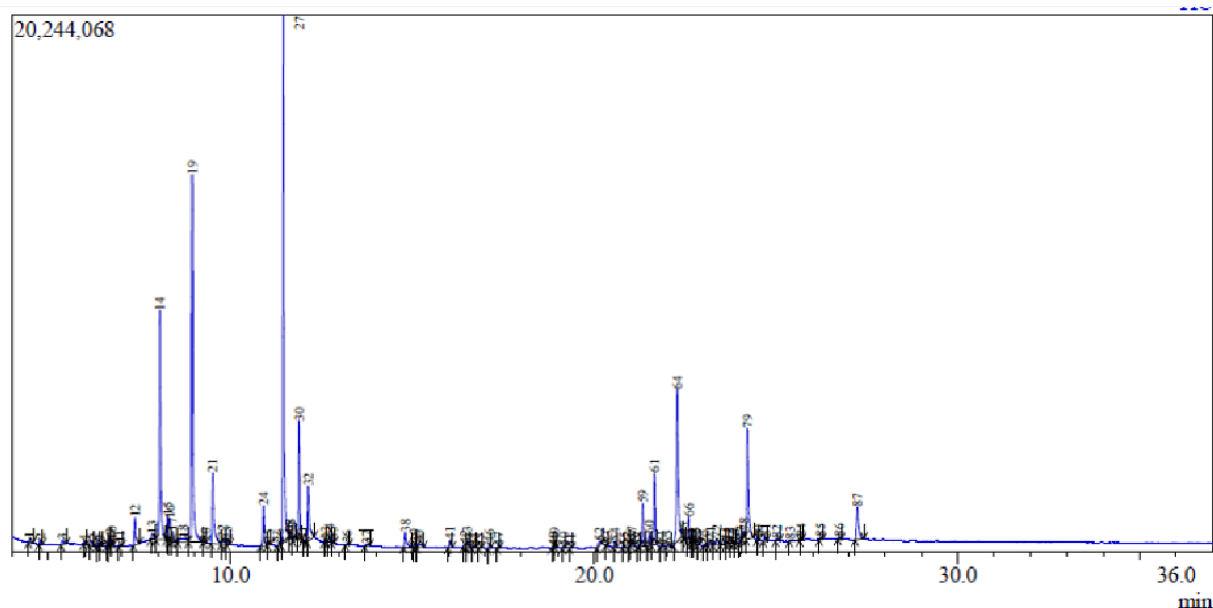


**Figure 6.** Severity of *A. alternata* Infection on Tomato and Potato Plants (8 Isolates)

### 3.4. CHEMICAL ANALYSIS OF *C. MACROCARPA* VOLATILE OILS

Gas chromatography-mass spectrometry (GC-MS) analysis of *C. macrocarpa* essential oil revealed a rich chemical composition comprising over 80 different compounds (Table 3, Fig. 7). The results indicated a clear predominance of monoterpenes, with 4-terpineol constituting 21.49% of the total components, followed by  $\gamma$ -terpinene at 15.89% and  $\alpha$ -terpinene at 10.37%, which collectively define the characteristic aroma of the oil. Significant quantities of fatty acid esters were also recorded, such as hexadecanoic acid ethyl ester (8.68%) and octadecanoic acid ethyl ester (6.15%). Other notable compounds included  $\alpha$ -terpinolene (3.99%),  $\beta$ -citronellol (3.25%), and citronellal (3.20%), as well as smaller percentages of camphor and D-limonene. This integrated profile of bioactive terpene derivatives, complemented by lipidic esters, underscores both the aromatic character and potential pharmacological properties of this unique essential oil.

In previous studies, the essential oils of Egyptian cypress were studied by Badawy et al. [53]. The data from this study show that monoterpenes are the dominant constituents of *Cupressus macrocarpa* oil. They reported the richness of the oil in 4-terpenol (20.29%) and other monoterpenes, which is consistent with our results. However, the relatively high proportion of sabinene (18.67) and citronellol (13.01) observed in our sample contrasts



**Figure 7.** GC-MS chromatogram of *C. macrocarpa* essential oil.

**Table 3.** Chemical compositions of the essential oil of *C. macrocarpa* leaves.

| RT    | Component   | Area %       | Molecular formula                              |
|-------|---|--------------|--|
| 7.37  | $\beta$ -myrcene  | 1.41         | C <sub>10</sub> H <sub>16</sub>                |
| 8.06  | $\alpha$ -Terpinene   | 10.37        | C <sub>10</sub> H <sub>16</sub>                |
| 8.27  | Benzene, methyl(1-methylethyl)- Cymene                                  | 1.20         | C <sub>10</sub> H <sub>14</sub>                |
| 8.34  | D-Limonene  | 1.04         | C <sub>10</sub> H <sub>16</sub>                |
| 8.40  | $\beta$ -Phellandrene   | 0.47         | C <sub>10</sub> H <sub>16</sub>                |
| 8.94  | $\gamma$ -Terpinene   | <b>16.75</b> | C <sub>10</sub> H <sub>16</sub>                |
| 9.51  | $\alpha$ -Terpinolene   | 3.99         | C <sub>10</sub> H <sub>16</sub>                |
| 9.78  | Linalool  | 0.35         | C <sub>10</sub> H <sub>18</sub> O              |
| 10.91 | Camphor   | 1.82         | C <sub>10</sub> H <sub>16</sub> O              |
| 11.45 | 4-Terpineol   | <b>25.73</b> | C <sub>10</sub> H <sub>18</sub> O              |
| 12.13 | Octadecanoic acid, ethyl ester  | 6.15         | C <sub>20</sub> H <sub>40</sub> O <sub>2</sub> |
| 21.33 | Succinic acid, di(3,7-dimethyloct-6-en-1-yl) ester                      | 2.02         | C <sub>24</sub> H <sub>42</sub> O <sub>4</sub> |
| 21.66 | Citronellal   | 3.20         | C <sub>10</sub> H <sub>20</sub> O              |
| 21.66 | Citronellal   | 3.20         | C <sub>10</sub> H <sub>18</sub> O              |
| 22.28 | Hexadecanoic acid, ethyl ester  | 8.68         | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> |
| 22.60 | Kaur-15-ene   | 1.02         | C <sub>20</sub> H <sub>32</sub>                |
| 27.24 | 1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS) Isooctyl phthalate | 2.06         | C <sub>24</sub> H <sub>38</sub> O <sub>4</sub> |
| –     | Other (< 0.1%)  | 10.47        | –  |
|       | Total identified  | 98.91        |  |

with their results, likely reflecting differences in plant parts or extraction conditions. In contrast, the essential oils of Egyptian cypress were studied by Sherif, et al. [54]. GC-MS analysis of the essential oil components of *C. macrocarpa* revealed D-limonene (38.00%) as the major component, followed by citral (9.72%), carveol (6.86%), and citronellal (5.35%). The differences in essential oil compositions could be due to several factors, such as geographical location, season, environmental conditions, nutritional status of the plants, and other factors.

### 3.5. ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL

The antifungal efficacy of 0.2%, 0.4%, and 0.6% *C. macrocarpa* essential oil was evaluated and compared with that of 0.1% difenoconazole against several fungal isolates (Table 4, Fig. 8). Both treatments demonstrated significant and variable inhibitory activity. The *C. macrocarpa* essential oil exhibited its highest mean inhibition against isolate *A. alternata* 97.5 ± 3.43% at concentration 0.6%, 66.4 ± 3.43%, and 83 ± 3.43% at 0.2 and 0.4 concentrations, respectively.

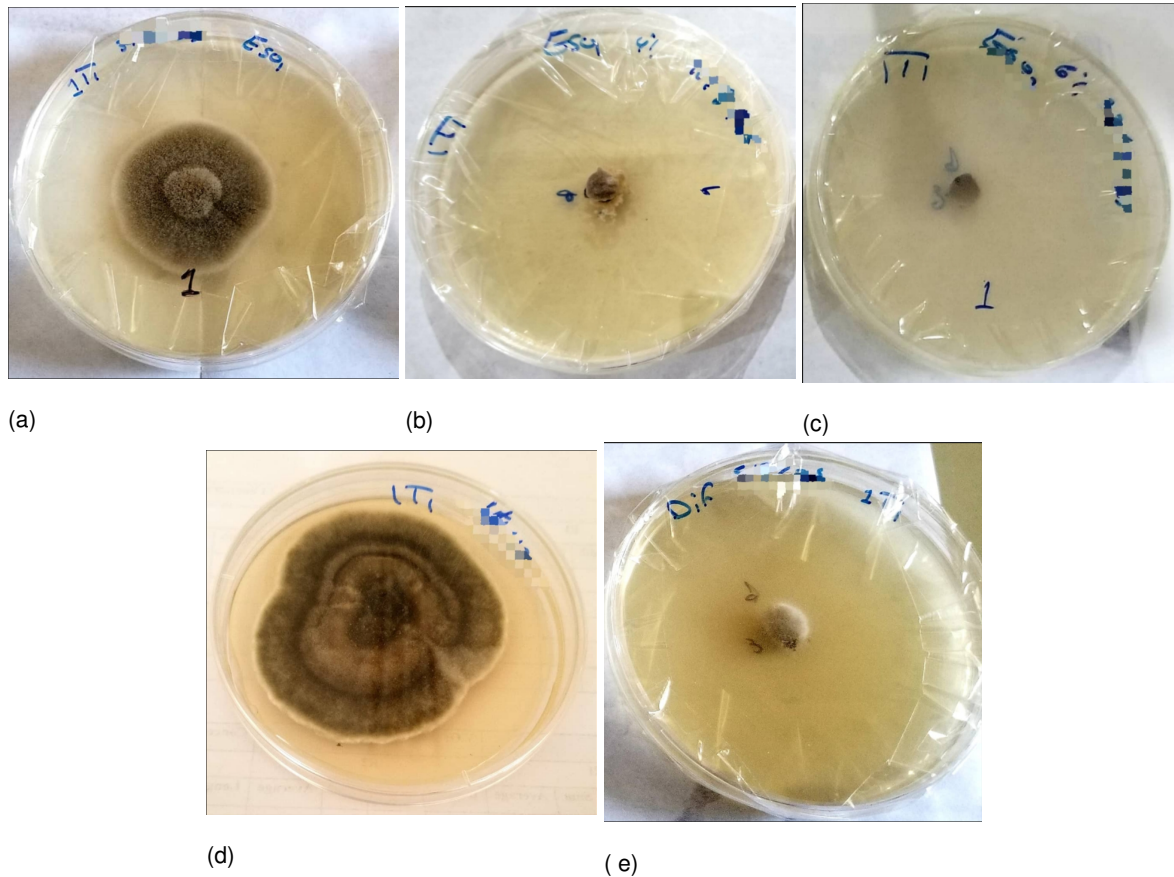
In contrast, difenoconazole displayed more consistent potency, with a 90 ± 2.52% inhibition rate.

**Table 4.** In vitro radial growth and inhibition of *A. alternata* isolate by *C. macrocarpa* Oil

| Treatment                | Concentration% | Radial growth *(mm) | Growth Inhibition %**     |
|--------------------------|----------------|---------------------|---------------------------|
| <i>C. macrocarpa</i> Oil | 0.2            | 2.4                 | 64.78 ± 3.43 <sup>c</sup> |
|                          | 0.4            | 1.4                 | 83 ± 3.43 <sup>ab</sup>   |
|                          | 0.6            | 0.8                 | 97.5 ± 3.43 <sup>b</sup>  |
| Difenoconazole           | 0.1            | 1.20                | 90 ± 2.52 <sup>a</sup>    |
| Control                  |                | 4.18                | 0.00                      |

\* Radial growth was measured for the isolate *A. alternata*, obtained from the microbial culture collection in Sana'a, Yemen.

\*\* Values of growth inhibition are presented as mean ± standard deviation (n = 3). Statistical comparisons were performed within a single column using Duncan's Multiple Range Test (DMRT) at  $P \leq 0.05$ . Means followed by different letters are significantly different ( $P \leq 0.05$ ), whereas those followed by the same letter are not significantly different.



**Figure 8.** Efficacy of *C. macrocarpa* essential oil and Difenoconazole on the mycelium growth of *A. alternata* isolate compared to untreated control in vitro. (a, b, c) *C. macrocarpa* essential oil at concentrations of (0.2%, 0.4%, and 0.6%), respectively. (d) Control and (e) Difenoconazole at a concentration of 0.1%.

The essential oil of *C. macrocarpa* exhibited significant antifungal activity against *A. alternata* and inhibited fungal mycelial growth. Therefore, the use of essential oils as antifungal agents is a promising approach for the management of early blight diseases in tomato and potato crops. Statistical analysis revealed a significant difference between all concentrations of *C. macrocarpa* (0.2%, 0.4%, and 0.6%) and 0.1% difenoconazole pesticide when compared to the control group. Moreover, a statistically significant difference ( $P < 0.05$ ) was observed between all *C. macrocarpa* concentrations and difenoconazole.

Dose-response analysis demonstrated a clear concentration-dependent inhibition pattern, culminating

in complete suppression of fungal growth at a minimum inhibitory concentration (MIC) of 6.0  $\mu\text{L/mL}$ . Despite the extensive body of literature documenting the antifungal properties of essential oils, studies specifically evaluating lemon cypress (*C. macrocarpa*) oil against *A. alternata* are scarce. Therefore, the present findings identify this oil as a potent yet underexplored botanical fungicide and contribute novel insights to the existing knowledge base on plant-based fungicides.

These results are strongly supported by recent studies. Kou et al. [55] demonstrated that *Artemisia capillaris* essential oil effectively inhibits *Alternaria* species by disrupting cellular ultrastructure, while Thambi et al. [56] reported the significant efficacy of *Monarda citriodora* oil



in controlling *Alternaria* leaf spots. Furthermore, our findings are consistent with those of Badawy et al. [53], who observed mycelial growth inhibition ranging from 50% to 95% for *C. macrocarpa* at 318 mg/L against the same pathogen, and Amri et al. [57], who reported 75.21% inhibition for *Cupressus sempervirens* at a concentration of 0.4%.

In addition, the observed antifungal activity is comparable to that reported by Kumar et al. [58], who achieved approximately 90% inhibition using difenoconazole (0.1%), thereby providing a benchmark that underscores the strong inhibitory potential of lemon cypress oil as a sustainable and eco-friendly alternative to conventional fungicides.

#### 4. CONCLUSION

This study identified *A. alternata* as the causal agent of early blight in tomato and potato crops in Sana'a Governorate, Yemen, and revealed considerable variability in morphology and pathogenicity among local isolates. The evaluation of *C. macrocarpa* volatile oil demonstrated remarkable antifungal efficacy, with inhibition rates comparable to or exceeding those of the synthetic fungicide difenoconazole. This study indicates that although maximum inhibition occurred at a 0.6% concentration, the 0.4% concentration is advised as the best formulation for both oils. This concentration offers superior efficacy (>83%) while markedly decreasing the expenses and dangers associated with elevated doses. The chemical composition of the oil, which is rich in monoterpenes such as 4-terpineol, provides a strong mechanistic basis for its activity. The current study explored the potential use of *Cupressus macrocarpa* volatile oil against *A. alternata*, and its compounds could be further investigated for the development of eco-friendly approaches for the control of early blight.

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