



---

*Research article*

## **Control of *Septoria tritici* in durum wheat: Genetic evaluation of seven traditional genotypes and assessment of antifungal potential of five plant-derived essential oils**

**Fethi Bnejdi<sup>1,2</sup>, Mourad Saadoun<sup>1</sup>, Abdelhamid Hajji<sup>1</sup>, Ines Jlassi<sup>1</sup>, Teyssir Bouattour<sup>1</sup>, Anis Ben Hsouna<sup>3,4</sup>, Miroslava Kačániová<sup>5,6</sup>, Sonia Marghali<sup>7</sup>, Mossadok Ben-Attia<sup>8</sup>, Wissem Mnif<sup>9</sup>, Angelo Maria Giuffrè<sup>10,\*</sup> and Safia El-Bok<sup>1</sup>**

<sup>1</sup> Laboratory of Biodiversity, Biotechnology, and Climate Changes, Faculty of Sciences of Tunis El Manar El Manar II, Tunis 2092, Tunisia; fethi.benejdi@issacm.u-sousse.tn (F.B.), hajji.abdelhamid@hotmail.com (A.H.), safia.elbok@fst.utm.tn (S.E.B.)

<sup>2</sup> Department of Biological Sciences, University of Sousse, Higher Institute of Agronomic Sciences of Chott-Mariem, 4042, Chott-Mariem, Sousse, Tunisia

<sup>3</sup> Laboratory of Biotechnology and Plant Improvement, Centre of Biotechnology of Sfax, B.P “1177”, Sfax 3018, 9, Tunisia; benhsounanis@gmail.com (A.B.H.)

<sup>4</sup> Department of Environmental Sciences and Nutrition, Higher Institute of Applied Sciences and Technology of Mahdia, University of Monastir, Monastir 5000, Tunisia

<sup>5</sup> Institute of Horticulture, Faculty of Horticulture, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia; miroslava.kacaniova@gmail.com (M.K.)

<sup>6</sup> School of Medical & Health Sciences, VIZJA University, Okopowa 59, 01 043 Warszawa, Poland

<sup>7</sup> Laboratory of Microorganisms and biomolecules actives LR03ES03, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunisia; sonia.marghali@fst.utm.tn (S.M.)

<sup>8</sup> Environment Biomonitoring Laboratory (LR01/ES14), Faculty of Sciences of Bizerte, University of Carthage, 7021 Zarzouna, Bizerte, Tunisia; mossadok.benattia@fsb.ucar.tn (M.B.A.)

<sup>9</sup> Department of Chemistry, College of Science, University of Bisha, P.O. Box 551, Bisha 61922, Saudi Arabia; w\_mnif@yahoo.fr (W.M.)

<sup>10</sup> Department AGRARIA, University of Studies “*Mediterranea*” of Reggio Calabria, 89124 Reggio Calabria, Italy; amgiuffre@unirc.it (A.M.G.)

\* **Correspondence:** Email: amgiuffre@unirc.it; Tel: +39(0)3277022840.

**Abstract:** Effective management of *Septoria tritici* blotch (STB) remains a major challenge in durum wheat-growing regions, particularly as *Triticum durum* production continues to suffer from increasing disease pressure. The reduced effectiveness of previously resistant cultivars highlights the urgent need to identify new genetic resources and alternative control strategies. This study evaluated both genetic resistance and biological control approaches to improve STB management. Seven Tunisian durum wheat genotypes, including six previously abandoned due to low productivity, were assessed for their response to STB. Analysis of variance of area under disease progress curve (AUDPC), transformed AUDPC, and percentage of infected leaf area revealed highly significant differences among genotypes, enabling clear discrimination of resistance levels. Across all disease parameters, mean comparisons showed that Beskri, Chetla, and Rommani exhibited consistently high tolerance to STB, whereas Chili and Swabaa Algiaa exhibited a moderate tolerance. In contrast, Jneh Kottifa and Karim were classified as susceptible genotypes, displaying the highest levels of disease severity and disease development. In parallel, the antifungal potential of six plant-derived essential oils was investigated using fumigation and contact bioassays. Essential oils derived from *Thymus capitatus*, *Rosmarinus officinalis*, *Eucalyptus camaldulensis*, *Artemisia herba-alba*, and *Cinnamomum bark* were evaluated at three concentrations (2, 5, and 10  $\mu$ L for fumigation assays and 20, 40, and 80  $\mu$ L for contact bioassays) to assess their antifungal activity. In fumigation assays, inhibition of mycelial growth varied depending on oil type and dose. Cinnamomum and Thymus oils achieved complete inhibition at 2  $\mu$ L, whereas Eucalyptus reached a maximum inhibition of 69% at 10  $\mu$ L. *Artemisia herba-alba* showed moderate activity, while *Rosmarinus officinalis* displayed the lowest effectiveness. In contact bioassays the antifungal activity assessed after 12 days, confirming significant inhibitory effects. Cinnamomum oil caused total inhibition at 20  $\mu$ L. Eucalyptus oil produced inhibition rates ranging from 63.5% to 100% at volumes between 20 and 80  $\mu$ L. *Artemisia herba-alba* exhibited a dose-dependent response, with inhibition between 32.92% and 67.46%, whereas Thymus oil showed limited activity, not exceeding 20.7%. The identification of STB-tolerant Tunisian durum wheat genotypes provides promising new sources of resistance that may offset the declining performance of previously reliable cultivars. Their integration into breeding programs, combined with plant-based antifungal agents, represents a valuable strategy for sustainable and long-term control of *Septoria tritici*.

**Keywords:** *Septoria tritici* Blotch; durum wheat; genetic resistance; essential oils; biological control; antifungal activity; plant-derived compounds; disease management

## 1. Introduction

*Triticum durum wheat* is one of the most widely cultivated and consumed cereal crops globally, providing approximately 20% of the protein consumed by the human population and serving as a staple food for nearly one-third of the world's population [1]. In Tunisia, durum wheat is the primary cultivated cereal crop, occupying more than half of the land allocated to cereal cultivation. In the Mediterranean region has been significantly affected by biotic and abiotic stress. Among the major diseases affecting wheat worldwide, *Septoria tritici* blotch (STB), caused by the fungal pathogen *Zymoseptoria tritici*, is one of the most destructive. It leads to significant yield losses and affects both the quantity and quality of grain production [2]. Under favorable conditions for the pathogen,

yield losses can exceed 50% in susceptible genotypes [3], resulting in billions of dollars in losses annually for major wheat-producing countries, both in terms of reduced yields and increased fungicide use [4]. Currently in Tunisia, no wheat genotype has demonstrated long-term stable resistance to STB. Genotypes that were previously considered resistant or moderately resistant are now exhibiting susceptibility, complicating breeding efforts aimed at genetic control of this disease. For example, the Tunisian cultivar *Salim*, which was registered in 2010 as STB-resistant, has since shown a loss of resistance. This erosion of resistance has not been widely discussed in the literature but has been noted in recent research reports [5]. Recent studies have shown that traditional landraces previously considered resistant to *Zymoseptoria tritici* may become susceptible over time due to the rapid evolutionary potential of the pathogen [6,7]. This suggests that the pathogen has undergone rapid evolution, likely due to its high genetic variability and sexual reproduction, which enable it to produce new virulent strains capable of overcoming host resistance genes. The aggressiveness of *Z. tritici* populations can vary by region and is influenced by environmental and climatic factors, further complicating resistance breeding [8].

Several challenges hinder the development of durable resistance in wheat. First, genetic resistance to STB is quantitatively inherited and governed by complex genetic mechanisms, including epistasis and maternal effects, making it difficult to fix desirable traits in breeding lines [9]. Second, the overuse of fungicides has led to the selection of more aggressive pathogen strains [10], and populations of STB have rapidly evolved resistance to many commonly used fungicides [11]. Third, the high rate of sexual recombination and frequent mutations within STB populations has resulted in significant genetic diversity, increasing the pathogen's ability to overcome both chemical and genetic controls [12].

In Tunisia, formerly resistant varieties, such as Maali and Salim, have lost their effectiveness, and STB pressure continues to intensify each year. Bnejdi et al. [13] emphasized that resistance to STB is strongly influenced by the aggressiveness of the pathogen isolate used in evaluation, recommending the use of the most virulent isolates in breeding programs to identify durable resistance. Furthermore, fungicide-based management of STB faces additional obstacles. Specific mutations conferring resistance to fungicides have been identified in STB populations, making chemical control increasingly unreliable. In addition, widespread fungicide use poses serious risks to human and animal health, as well as the environment [14].

The development of sustainable strategies for the management of STB requires the integration of complementary approaches, including the deployment of resistant wheat genotypes and the use of environmentally friendly biocontrol agents. In this context, essential oils (EOs) exhibiting antifungal activity against *Zymoseptoria tritici* may represent a promising tool to complement host plant resistance within integrated disease management programs, thereby reducing reliance on synthetic fungicides. Given these challenges, alternative approaches are needed. The objective of this study was to (i) evaluate the level of resistance to STB in selected old Tunisian durum wheat genotypes and (ii) assess the antifungal activity of six plant-derived EOs against STB using fumigation and contact bioassays.

## 2. Materials and methods

### 2.1. Evaluation of wheat genotypes for resistance to STB

#### 2.1.1. Plant materials and experimental design

The modern durum wheat variety Karim and six traditional genotypes (i.e., Chili, Chettla, Swabaa Algiaa, Rommani, Biskri, and Jneh Khottifa), which had been abandoned for many years due to low productivity, were evaluated for their tolerance to natural infection by *Zymoseptoria tritici* (causal agent of STB). The trial was conducted at the experimental station in Tunis during the 2020–2021 growing season. The 2020–2021 season at Tunis El Manar was characterized by moderate rainfall (350 mm), mild winter temperatures, and relative humidity ranging from 50% to 75%, conditions generally conducive to *Zymoseptoria tritici* infection and STB development. These environmental conditions likely contributed to the observed differences in disease severity among durum wheat genotypes. Genotypes were planted in a randomized complete block design (RCBD) with two replications.

#### 2.1.2. Disease assessment and severity scoring

STB incidence on each genotype was recorded using the Zadoks growth stage scale [15]. Disease severity was evaluated using the double-digit scale (00–99), a modified version of the Saari–Prescott severity scale developed for assessing foliar diseases in wheat [16,17]. In this scale, the first digit (D1) reflects the vertical progress of the disease on the plant. A value of 0 indicates symptoms confined to the basal leaves, while a value of 9 denotes symptoms present on leaves, sheaths, glumes, and awns. The second digit (D2) represents disease severity as the percentage of leaf area covered with lesions bearing pycnidia, ranging from 0 (no infection) to 9 (fully susceptible).

Disease severity was calculated using the following formula:

$$\text{Severity (\%)} = (D1 / 9) \times (D2 / 9) \times 100$$

#### 2.1.3. Estimation of area under disease progress curve (AUDPC)

To quantify the progression of STB over time, the AUDPC was calculated for each genotype, following the method described by Castro and Simón [18] and Chartrain et al. [19]. The AUDPC provides a cumulative measure of disease intensity and epidemic development throughout the crop cycle.

$$AUDPC = \sum_{i=1}^{n-1} \left( \frac{Y(i+1) + Y_i}{2} \right) (t(i+1) - t_i)$$

where:

- $n$  = total number of disease assessments;
- $Y_i$  = disease severity (%) at the  $i$ th observation;
- $t_i$  = time (days) at the  $i$ th observation.

This method allows for a more comprehensive evaluation of genotypic tolerance by capturing both the intensity and rate of disease development [20,21].

A significant linear relationship between the standard deviation and mean AUDPC values ( $y = ax + b$ ) revealed non-homogeneous variances across genotypes. Consequently, T-AUDPC values were

estimated using the logarithmic transformation proposed by Kleczkowski,  $T\text{-AUDPC} = \log(x + a/b)$ , where  $x$  represents the AUDPC value and  $a$  and  $b$  are the regression parameters. This transformation was performed to stabilize variances and ensure the validity of subsequent statistical comparisons among genotypes.

## 2.2. Antifungal activity of plant EOs against *Septoria tritici*

### 2.2.1. Plant material collection and essential oil extraction

This section employed five abundantly available plant-based spices. Leafy parts of *Thymus capitatus*, *Artemisia herba-alba* and *Rosmarinus officinalis* were collected between September and October from Jbel Aammar, located in the region of Tunis. Leaves of *Eucalyptus camaldulensis* were harvested from the campus of the Faculty of Sciences of Tunis, and bark of *Cinnamomum Bark* was obtained from a local farmer. The collected plant materials were air-dried in the shade for two weeks, then ground into a fine powder and stored in a cool, dry environment until further use.

EOs were extracted from the plant materials using a hydro-distillation process for three to five hours with a Clevenger-type apparatus (Idam Cam, Ankara, Turkey). A total of 100 g of dried plant material was distilled in distilled water for three hours. The extracted EOs were then separated, dried over anhydrous sodium sulfate, and transferred to sealed vials. For short-term storage, the EOs were kept in dark bottles at 4 °C, while long-term storage was conducted at –20 °C.

### 2.2.2. In vitro antifungal activities on Mycelial growth

#### 2.2.2.1. Contact bioassay

The antifungal properties of EOs extracted from five species (i.e., *Thymus capitatus*, *Rosmarinus officinalis*, *Eucalyptus camaldulensis*, *Artemisia herba-alba*, and *Cinnamomum Bark*) were evaluated based on their effects during the contact phase on the mycelial development of STB. To prepare homogeneous emulsions for testing, the EOs were dissolved in distilled water sterilized with 0.1% (v/v) Tween 20 (Sigma Aldrich, Steinheim, Germany). These emulsions were incorporated into sterilized PDA medium at 40 °C to obtain three concentrations: 20, 40, and 80 µL/mL. A 0.1% (v/v) Tween 20 solution was used as the control.

The mixtures were immediately poured into 90 mm diameter Petri dishes at 20 mL per plate. Once the media solidified, 5 mm diameter plugs of actively growing STB culture, taken from the periphery of fresh cultures, were aseptically transferred onto each plate. Each concentration was tested in triplicate. The inoculated plates were sealed with parafilm and incubated in darkness at  $22 \pm 2$  °C for 11 days.

Mycelial growth was measured every two days, with observations ceasing when the control plates were fully covered by fungal colonies. The percentage of mycelial growth inhibition was calculated using the formula

$$\text{Inhibition (\%)} = [(C - T)/C] \times 100$$

where  $c$  is the mean diameter of mycelial growth in the control and  $t$  is the mean diameter in the treatment.

#### 2.2.2.2. Fumigation bioassay

A highly virulent isolate of *Zymoseptoria tritici* was collected from naturally infected leaves of the susceptible durum wheat cultivar Karim at the experimental station of the Faculty of Sciences of Tunis (Tunisia), where STB occurs regularly under natural field conditions. The isolate was purified by transferring a single pycnidiospore to potato dextrose agar (PDA) and its identity was confirmed based on the characteristic morphology of pycnidia and pycnidiospores using standard taxonomic criteria. The pure culture was maintained on PDA and stored at 22 °C until use. Sterile PDA was poured into 9-cm diameter sterile Petri dishes. After solidification, the plates were kept inverted. A sterilized 9-cm diameter filter paper was placed on the interior side of each dish cover. Four volumes (0, 2, 5, and 10 µL) of each of the six previously mentioned plant EOs were aseptically injected into the center of the filter paper. Then, fungal mycelial disks of 5 mm diameter, taken from actively growing cultures, were inoculated in the center of the PDA-containing plates. The plates were tightly sealed with parafilm and incubated at 27 °C for 2–3 weeks. Colony diameters were measured weekly. The inhibitory effects of the EOs against STB were assessed by measuring the diameter of fungal colonies after seven days of incubation and comparing these to the control plates.

To quantify the antifungal effects of the EOs on *Septoria tritici* mycelial growth, the percentage of growth inhibition was calculated and expressed as percent according to the formula described by Farzaneh et al. [22]:

$$\text{Inhibition (\%)} = [(C-T)/C] \times 100$$

#### 2.2.3. Gas chromatography-mass spectrometry (GC-MS) analysis

Each essential oil's volatile components were examined using GC-MS. A gas chromatograph (Agilent 7890B; Agilent Technologies Inc., Santa Clara, CA, USA) was used to process them. Using a GC-MS system made up of a GC instrument (Agilent 6890N) equipped with a mass selective detector (Agilent 5973N), the essential oil compositions of the plant materials were determined. The components were distinguished using an HP Innnowax Capillary Column (60.0 m x 0.25 mm x 0.25 µm film thickness) (Agilent 19091S-433). By comparing the mass spectra of each peak with the information provided by the National Institute of Standards and Technologies (NIST) Mass Spectra Library, separated chemical compounds were identified using gas chromatography. The EOs obtained from extraction were dried with anhydrous sodium sulfate (Merck, Darmstadt, Germany) stored then at 4 °C until analysis. Gas chromatography (GC) analyses were carried out using with Hewlett-Packard 6890 GC Flame Ionization Detector (FID). Chromatographic separation was performed on an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.4 mL/min. The oven temperature program was as follows: 45 °C for 5 min, ramped at 3 °C/min to 220 °C, with a final hold of 10 min. The injector and detector temperatures were maintained at 220 °C and 250 °C, respectively. Injection was carried out automatic mode. Samples (0.5 µL) of the oil solution in hexane (1:100, v/v) were injected (1 µL) in split less mode using helium as the carrier gas. Peak areas and retention times were determined by electronic integration.

#### 2.3. Statistical analysis

All statistical analyses and graphs in this study were performed using the R statistical

package (<http://www.r-project.org/>; R-4.3.2). Before analysis, Kleckowski's transformation [23] was applied to normalize the distribution of data or to make means independent of variances and means were compared using Tukey's test.

### 3. Results

#### 3.1. Evaluation of seven durum wheat genotypes against tolerance to STB

Analysis of variance (One-way ANOVA) of the three assessed traits revealed a highly significant varietal effect on resistance to *Zymoseptoria tritici* (data not shown). The coefficient of determination ( $R^2$ ) ranged between 70% and 98%, indicating that the model was reliable and the experiment was well conducted.

Estimates of STB incidence across the seven genotypes are shown in Figures 1–3. According to Tukey's test, significant differences in STB incidence were observed between genotypes for all three traits: percentage of infected leaf area, AUDPC, and transformed AUDPC (TAUDPC).

The percentage of necrotic surface on the flag leaf varied from 0 to 90% among varieties (Figure 4). The highest STB incidence was observed in genotypes Jneh el-Khottifa and Swabaa Aljia, which showed little significantly reduced necrotic surface compared to the susceptible variety Karim. Genotype Chili exhibited an intermediate response, characterized by a moderate level of leaf necrosis, indicating partial susceptibility to STB. In contrast, genotypes, such as Chetla, Beskri, and Rommani, displayed a high level of partial resistance, with the percentage of necrotic flag leaf surface ranging from 0% to 20%.

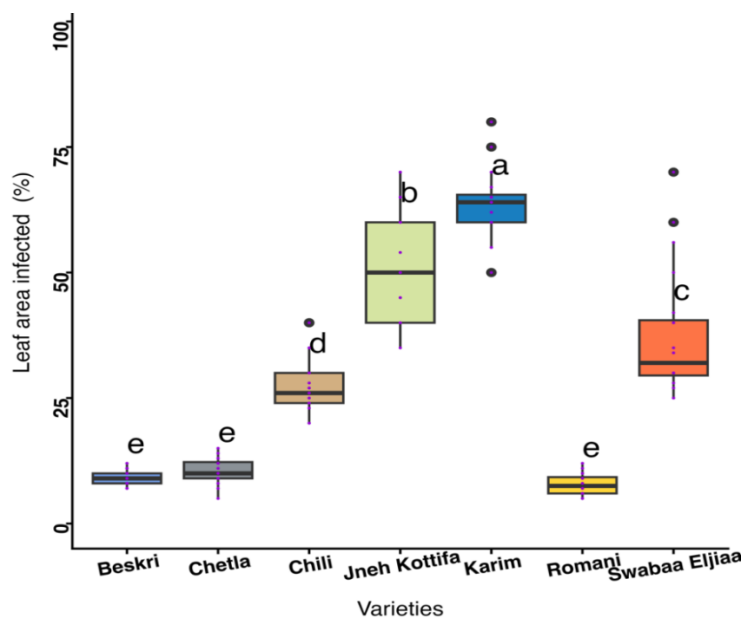
The AUDPC is widely recognized as a robust indicator of disease development and is commonly used to distinguish between tolerant and susceptible genotypes to foliar diseases. In the current study, the highest AUDPC values were recorded for Karim 80 and Jneh el-Khottifa, with values of 1987 and 2557, respectively. Moderate AUDPC scores were observed for Swabaa Aljiaa and Chili, with values of 1133 and 847. In contrast, the lowest AUDPC scores were found in Chetla (364), Beskri (222), and Rommani (212). However, a known issue with AUDPC is the correlation between the mean and variance, which can potentially distort analysis results. Therefore, Kleckowski's transformation was applied to the data, as shown in Figure 3. The resulting classification of varieties was consistent with that based on raw AUDPC values. The genotypes were grouped into three distinct categories:

Group 1: Karim and Jneh el-Khottifa (high susceptibility).

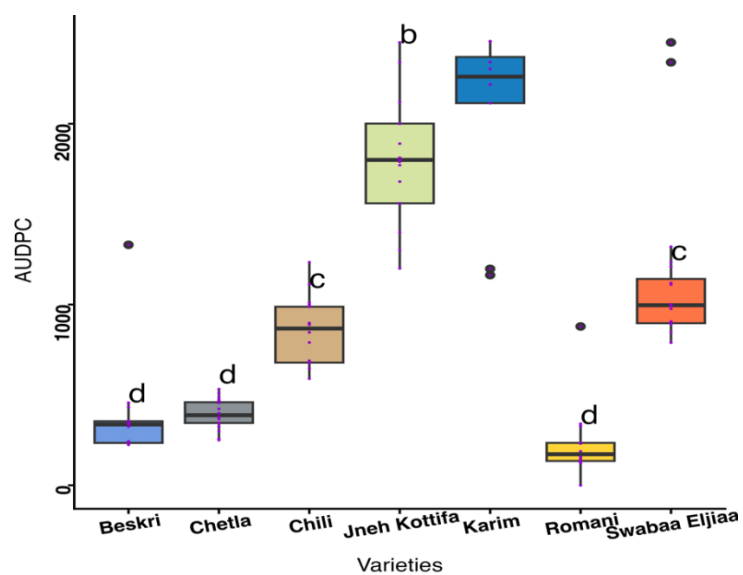
Group 2: Swabaa Aljiaa and Chili (moderate tolerance).

Group 3: Chetla, Beskri, and Rommani (high tolerance).

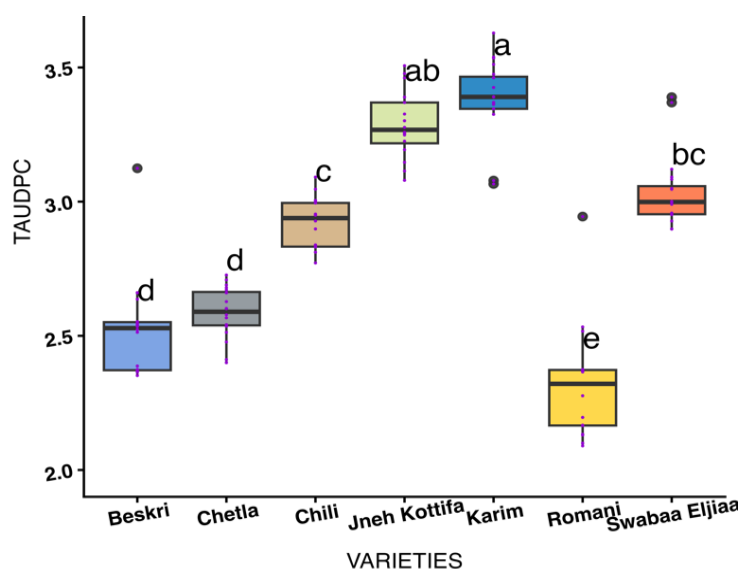
Boxplots of the three traits revealed that TAUDPC exhibited a more symmetric distribution compared to AUDPC, as shown by the median, minimum, and maximum values. This suggests that TAUDPC data followed a more normal distribution. These results highlight the existence of significant genetic diversity among the examined genotypes in terms of their tolerance to STB. Consequently, the observed variation provides a valuable basis for selecting desirable genotypes for breeding programs targeting STB resistance. Similar findings were reported in other studies. It is important to note that tolerance to *Z. tritici* may not be durable over time. In Tunisia, for instance, previously tolerant varieties, such as Maali and Salim, have lost their resistance.



**Figure 1.** Boxplot of percentage of the necrotic surface of flag area of the seven genotypes (Means followed by the same letter within boxplot were not significantly different at  $P < 0.05$ ).



**Figure 2.** AUDPC boxplot STB of seven Tunisian varieties (Means followed by the same letter within each column were not significantly different at  $P < 0.05$ ).



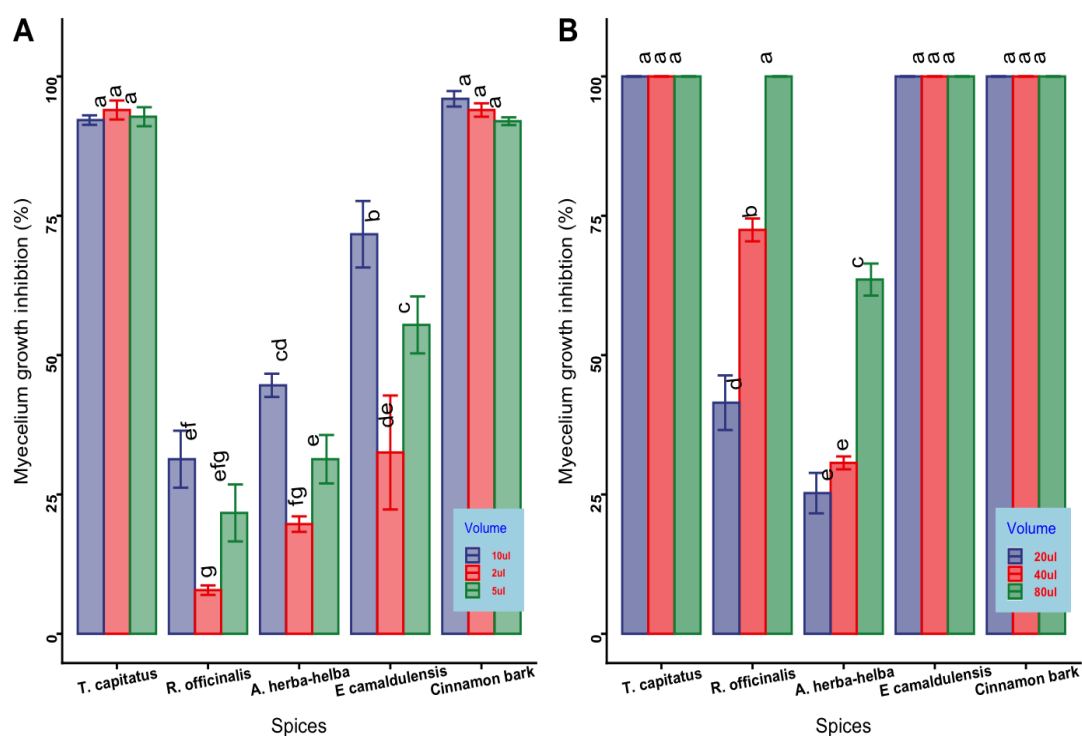
**Figure 3.** T-AUDPC boxplot STB of seven Tunisian varieties (Kleckowski's transformation) (Means followed by the same letter within each column were not significantly different at  $P < 0.05$ ).

| Photo | Genotype     | Symptoms caused by <i>septoria tritici</i>        |
|-------|--------------|---------------------------------------------------|
|       | Beskri       | No visible symptoms                               |
|       | Chetla       | Leaf yellowing or physiological symptoms          |
|       | Rommani      | 5–10% chlorotic and/or necrotic leaf area         |
|       | Chili        | 10–20% chlorotic and/or necrotic leaf area caused |
|       | Swabaa Eljia | 20–40% chlorotic and/or necrotic leaf area        |
|       | Jneh kottifa | 40–60% chlorotic and/or necrotic leaf area        |
|       | karim        | 60–90% chlorotic and/or necrotic leaf area        |

**Figure 4.** Representative symptoms of *Zymoseptoria tritici* infection on flag leaves of seven durum wheat genotypes, illustrating increasing percentages of chlorotic and/or necrotic leaf area.

### 3.2. Results of in vitro fumigation and contact antifungal activity

Results of fumigation and contact antifungal activity of the six EOs (*Thymus capitatus*, *Cinnamomum Bark*, *Artemisia herba-alba*, *Rosmarinus officinalis*, and *Eucalyptus camaldulensis*) against *Septoria tritici* were reported in Figure 5.

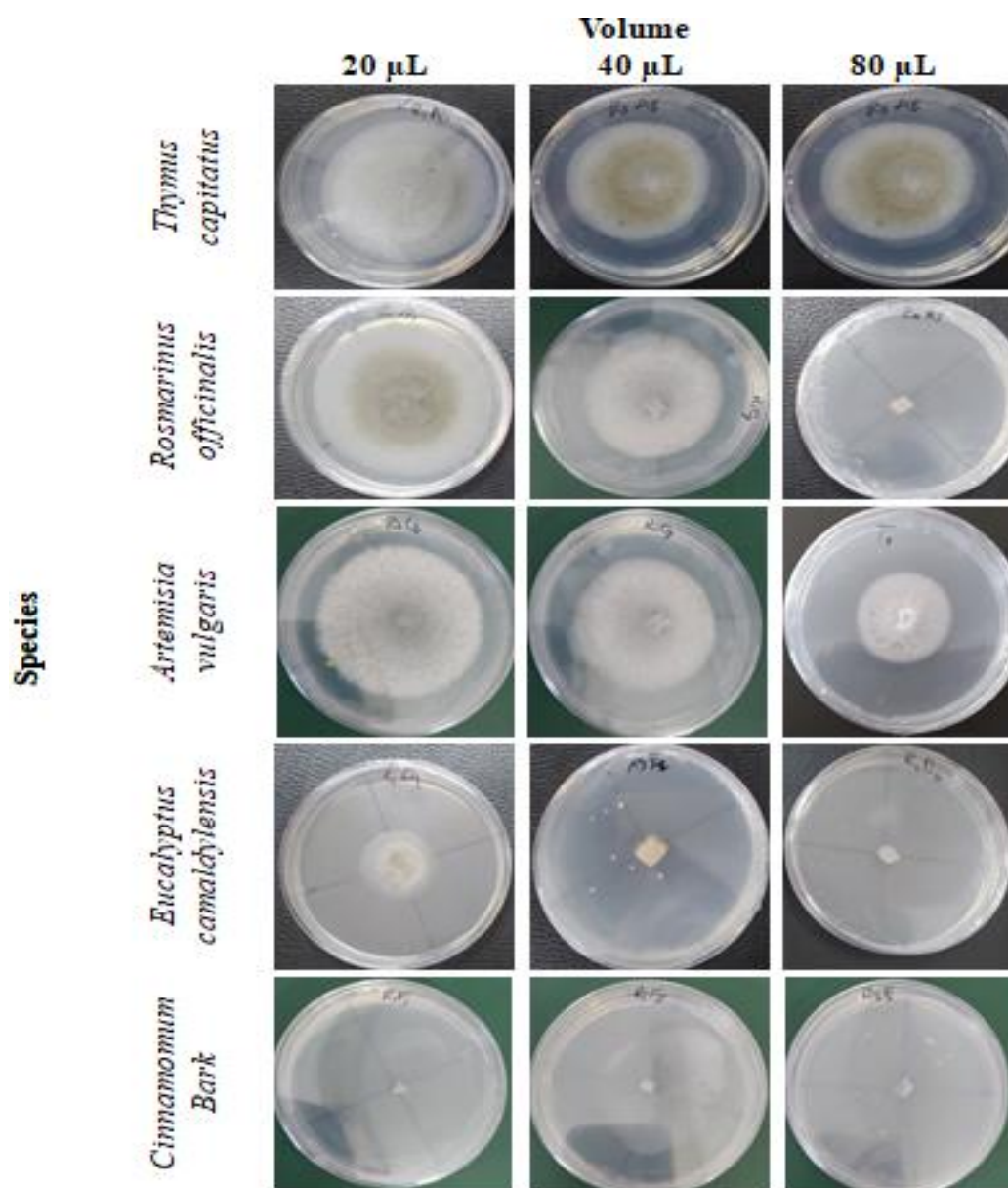


**Figure 5.** Percentage inhibition (%) of STB at different concentrations of five spice oils. (A) Contact bioassay; (B) fumigation bioassay. (Different letters indicate significant differences among combinations [spice-oil-concentration]).

Results of statistical analysis indicated that percent of inhibition depended upon method, species (plant EOs) and EOs concentration. In fact, in fumigation bioassay assessing antifungal activity, EOs of *Thymus capitatus* and *Cinnamomum Bark* demonstrated complete inhibition (100%) of *Septoria tritici* at a dose of 2 µL. In comparison, *Eucalyptus* EOs exhibited moderate to strong antifungal effects, with inhibition rates of 32.83%, 56.02% and 71.99% at concentrations of 2, 5, and 10 µL, respectively. Conversely, *Rosmarinus officinalis* and *Artemisia herba-alba* EOs showed relatively low antifungal activity, with maximum inhibition levels of 31.92% and 44.98%, respectively.

Similarly for contact bioassay, statistical analysis revealed that the inhibition of mycelial growth varied depending on both the type of EOs and the applied volume. Notably, *Cinnamomum bark* EOs resulted in complete inhibition (100%) of mycelial growth at the lowest tested volume (20 µL) (Figure 6). In the case of *Eucalyptus* EOs, inhibition ranged from 63.05% to 100% at volumes of 20 and 40 µL, respectively. For *Rosmarinus officinalis*, inhibition ranged from 7.42% at 20 µL to 100% at 80 µL, highlighting a dose-dependent effect. *Thymus capitatus* EOs exhibited the lowest antifungal activity, with inhibition not exceeding 20.7% at any tested volume. Overall, the inhibition rate was shown to be volume-dependent and ranged from 32.93% to 67.46% across the different EOs. These findings demonstrate that all five EOs tested exhibited some degree of inhibitory effect on the mycelial

growth of *S. tritici*, ranging from 8.4% (Thyme, 20  $\mu$ L) to 100% (Eucalyptus and Cinnamon).



**Figure 6.** Antifungal efficacy of EOs against *Septoria tritici* in a contact bioassay at three volumes (20, 40, and 80  $\mu$ L).

In contrast to the fumigation method, the results demonstrated that *Eucalyptus camaldulensis* essential oil exhibited strong contact antifungal activity, achieving 100% inhibition of *Zymoseptoria tritici* mycelial growth at 40  $\mu$ L across all tested volumes. A similar trend was observed for *Cinnamomum Bark*, which also resulted in complete inhibition (100%) at all tested volumes. Conversely, *Thymus capitatus* essential oil showed significantly weaker antifungal activity in contact assays compared to its strong effect in fumigation, with an average growth inhibition ranging from only 8.4% to 20.7%.

*Artemisia herba-alba* and *Rosmarinus officinalis* EOs exhibited moderate antifungal effects, with average inhibition ranging from 32.9% to 67.4% for *Artemisia* and 7.4% to 100% for *Rosemary*, depending on the volume applied. These findings indicate that *Cinnamomum* and *Eucalyptus* EOs

were the most effective in inhibiting the mycelial growth of *Z. tritici* in contact conditions.

The results of the statistical analysis presented in Figures 1 and 2 clearly demonstrate that the two EOs tested (i.e., *Cinnamon* bark and *Thymus capitatus*) exhibited complete inhibition according to both methods applied. Therefore, *Eucalyptus camaldulensis* was more efficient based in fumigation method and showed absolute toxicity (100% growth inhibition) against the tested fungus at the concentration of 20  $\mu$ L. For the other EOs, moderate fungitoxicity was observed using both methods. However, we noted that antifungal activities increased with increasing concentrations of plant EOs. Finally based in the two methods, the EOs of *Rosmarinus officinalis*, *Artemisia herba-alba*, and *Eucalyptus camaldulensis* demonstrated a clear concentration-dependent antifungal effect, indicating that increasing doses enhance their inhibitory efficacy against fungal pathogens. In conclusion, the efficacy of cinnamon oil was independent upon the two used methods. Since the evaluation of this EOs in vivo experiment was recommended for further application of this EOs as an ecofriendly biologic control. For volatile and no volatile fraction, cinnamon oil revealed with a pertinence antifungal activity against *Septoria tritici*.

### 3.3. Chemical composition of EOs

The chemical composition of the three most effective EOs (i.e., *Thymus capitatus*, *Cinnamomum Bark*, and *Eucalyptus camaldulensis*) was determined by GC-MS, and the results are presented in Tables 1–3. For *Thymus capitatus* EOs, a total of eight major compounds were identified, with carvacrol being the predominant component, accounting for 85% of the total composition. In the case of *Cinnamomum Bark* EOs, seven main compounds were identified, with cinnamaldehyde as the principal constituent, representing 97% of the EOs (Table 3). The EOs of *Eucalyptus camaldulensis* revealed 15 major components, with 1,8-cineole and camphor (bornanone) being the most abundant, accounting for 48.35% and 21.63% of the composition, respectively.

**Table 1.** Chemical compositions of EO *Thymus capitatus*.

| Peaks | Component              | RT (min) | RC (%) |
|-------|------------------------|----------|--------|
| 1     | $\alpha$ -Terpinene    | 10.21    | 0.61   |
| 2     | p-Cymene               | 10.43    | 5.23   |
| 3     | $\gamma$ -Terpinene    | 11.41    | 2.21   |
| 4     | Linalol                | 12.54    | 1.11   |
| 5     | Terpinène-4-ol         | 14.57    | 0.75   |
| 6     | Carvacrol              | 17.64    | 84.98  |
| 7     | Caryophyllene          | 20.28    | 4.13   |
| 8     | Oxyde de caryophyllene | 23.62    | 0.95   |

\*Note: RT(min), retention time (min); RC, relative concentration (%) of each identified compound calculated from the GC-MS peak area normalization.

**Table 2.** Chemical compositions of *Cinnamomum Bark* EO.

| Peaks | Component           | RT(min) | RC (%) |
|-------|---------------------|---------|--------|
| 1     | Cinnamaldehyde      | 10.212  | 97.21  |
| 2     | $\alpha$ -Copaene   | 10.436  | 0.35   |
| 3     | Cinnamyl acetate    | 11.413  | 0.61   |
| 4     | $\alpha$ -Muurolene | 12.54   | 0.47   |
| 5     | 1S.cis-Calamenene   | 14.571  | 0.47   |
| 6     | $\tau$ -Muurolol    | 17.646  | 0.53   |
| 7     | $\alpha$ -Cubebene  | 20.282  | 0.33   |

\*Note: RT(min), retention time (min); RC, relative concentration (%) of each identified compound calculated from the GC–MS peak area normalization.

**Table 3.** Chemical compositions of EO *Eucalyptus camaldylensis*.

| Peaks | Component            | RT (min) | RC (%) |
|-------|----------------------|----------|--------|
| 1     | $\alpha$ -Pinene     | 7.80     | 7.29   |
| 2     | Camphene             | 8.22     | 3.22   |
| 3     | $\beta$ -Pinene      | 9.045    | 1.13   |
| 4     | $\beta$ -Myrcene     | 9.503    | 0.53   |
| 5     | $\alpha$ -Terpinene  | 10.216   | 0.33   |
| 6     | p-Cymene             | 10.44    | 1.36   |
| 7     | 1,8-Cineole          | 10.628   | 48.35  |
| 8     | $\gamma$ -Terpinene  | 11.417   | 0.37   |
| 9     | $\beta$ -Linalool    | 12.544   | 0.63   |
| 10    | Bornanone or Camphor | 13.704   | 21.63  |
| 11    | Bornéol              | 14.28    | 8.18   |
| 12    | 4-Terpineol          | 14.575   | 1.06   |
| 13    | $\alpha$ -Terpineol  | 14.912   | 4.02   |
| 14    | Bornyl acetate       | 17.222   | 0.36   |
| 15    | trans-Caryophyllene  | 20.279   | 1.46   |

\*Note: RT(min), retention time (min); RC, relative concentration (%) of each identified compound calculated from the GC–MS peak area normalization.

## 4. Discussion

### 4.1. Evaluation of seven durum wheat genotypes against tolerance to STB

Mean disease incidence, as measured by AUDPC, TAUDPC, and the percentage of infected leaf area showed similar results that varied significantly among the evaluated varieties. The lowest STB incidence across all three traits was observed in the three traditional varieties (i.e., Chetla, Beskri, and Romani), highlighting the potential of older genotypes as valuable sources of resistance genes. The significant phenotypic variation observed in the present study is consistent with the quantitative nature of STB resistance. Supporting this, a recent genome-wide association study by Binalf et al. [24] identified 26 environment-specific loci significantly associated with STB resistance ( $P < 0.001$ ), primarily located on chromosomes 7B and 3A, thereby confirming that phenotypic resistance

assessed by AUDPC is under complex genetic control involving multiple loci. The identification of resistance alleles in traditional varieties, such as Chetla, Beskri, and Romani, further supports the value of genetic resource conservation in crop improvement.

The genetic variability observed among the tested genotypes increases the chance of successfully transferring resistance traits into high-yielding cultivars through breeding programs. Therefore, several considerations should be emphasized for breeders aiming to improve STB resistance. First, the high genetic diversity within *Z. tritici* populations must be taken into account. Linde et al. [25] emphasized that the high genetic variability within *Z. tritici* populations makes the durability of resistance genes uncertain, posing a significant challenge for breeding programs. Other studies have also reported highly significant differences in AUDPC values among genotypes [26]. The pathogen's aggressiveness can vary significantly among strains, which directly influences host-pathogen interactions. Multiple studies have shown that the genetic basis of plant resistance can differ depending on the aggressiveness of the infecting isolate. For example, Bnejdi et al. [13] demonstrated that resistance to less aggressive isolates could be explained by an additive-dominance model, whereas for more aggressive isolates, epistatic interactions played a more significant role in inheritance. Similarly, Alouane et al. [27] mapped a major QTL associated with aggressiveness variability, highlighting that the inheritance of pathogen aggressiveness is itself genetically controlled and varies between strains.

Second, evaluating the performance of the moderately and highly tolerant genotypes in different climatic regions is essential to identify stable and broadly resistant varieties. Multi-environment testing helps in understanding genotype  $\times$  environment interactions, which are crucial for the development of durable resistance. For instance, Kassie et al. [28] evaluated elite spring wheat genotypes across multiple locations in northwestern Ethiopia and found that some genotypes consistently exhibited resistance across all environments, confirming the necessity of such trials for effective cultivar selection.

In conclusion, integrating traditional genetic resources, understanding pathogen variability, and conducting multi-environment evaluations are essential components of breeding programs aiming to improve resistance to *Z. tritici* in wheat.

#### 4.2. Evaluation of *in vitro* fumigation and contact antifungal activity and the role of essential oil chemical composition

While the identification of resistant durum wheat genotypes represents a central key of sustainable STB management, host resistance alone may not provide durable protection due to the high genetic variability and adaptive capacity of *Zymoseptoria tritici*. Therefore, integrating resistant cultivars with environmentally friendly disease control measures, such as EO-based biofungicides, may enhance disease suppression and reduce dependence on synthetic fungicides. Such a combined strategy could contribute to a more resilient and sustainable management of STB under diverse climatic conditions. Currently, eco-friendly plant protection strategies aligned with sustainable agriculture principles are increasingly sought after [29]. In this context, biological control based on plant-derived products represents a promising complementary approach for the sustainable management of wheat diseases. EOs, due to their natural origin, biodegradability, and broad antimicrobial spectrum, have emerged as strong candidates for alternative disease control strategies. The antifungal activity observed in the present study is consistent with the findings of Allagui et al. [30], who evaluated antifungal activity of thirty EOs to control pathogenic fungi. Their results demonstrated significant inhibition of mycelial growth of the majority of tested pathogens, supporting the antifungal

potential of plant EOs confirmed in our study. Among the six tested oils, cinnamon EO showed the most pronounced antifungal activity in both fumigation and contact assays. Similar results were reported by Zheng et al. [31], who found that cinnamon bark and cinnamon oils exhibited the strongest antifungal effects against *Villosiclava virens* among 18 plant EOs tested. Chromatographic analyses reported in several studies, including Ranasinghe et al. [32], indicate that cinnamon EO is predominantly composed of cinnamaldehyde (often exceeding 50%), along with minor constituents, such as cinnamyl acetate and  $\beta$ -phellandrene, compounds strongly associated with its antimicrobial activity. The antifungal mechanism of cinnamaldehyde has been attributed to disruption of fungal cell membrane integrity and interference with essential cellular processes (Denkova-Kostova et al., [33]). Numerous studies have confirmed the broad antifungal spectrum of cinnamon EO against pathogens, such as *Aspergillus niger*, *Fusarium sambucinum*, *Aspergillus flavus*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Phomopsis viticola*, and *Rhizopus stolonifera* [34,35]. More recently, Liu et al. [36] demonstrated that cinnamaldehyde exhibited the lowest minimum inhibitory concentration (MIC) against *A. flavus* and *Penicillium citrinum* and significantly altered key cellular functions, reinforcing its role as the primary active compound. Thyme EOs also demonstrated remarkable antifungal activity. Several studies have reported the strong inhibitory effect of *Thymus capitatus* against various fungal strains. Omidbeygi et al. [37] showed that *Thymus capitatus* EO effectively inhibited *A. flavus*. Similarly, King et al. reported that *Thymus capitatus* exhibited the strongest fumigant antifungal activity among 10 plant EOs tested. These results corroborate those of Benoutman et al. [38] and Tabti et al. [34], who demonstrated high antifungal activity of *Thymus capitatus* against multiple fungal strains. The antifungal efficacy of thyme EO is mainly attributed to its richness in phenolic monoterpenes, particularly carvacrol and thymol. According to Pinto et al. [39] and Pinto et al. [40], Thymus EOs rich in these phenolic compounds exhibit strong antifungal properties. GC-MS analysis performed by Benoutman et al. [38] identified carvacrol as the predominant compound in *Thymus capitatus* EO. Mechanistically, carvacrol and thymol disrupt fungal membranes, increase permeability, cause cytoplasmic leakage, and reduce ergosterol content [41]. Socović et al. [42] further demonstrated that *Thymus vulgaris* EO rich in thymol (48.9%) showed very strong antifungal activity. Chemotype also plays a key role in antifungal potency. Ben Jabeur and Hamada [43] reported that the carvacrol chemotype of *Thymus capitatus* exhibited stronger antifungal activity than the thymol chemotype, attributing this difference to higher phenolic content. Similarly, Maissa and Walid [44] concluded that carvacrol-rich EO had more pronounced antifungal effects than thymol-rich EO, confirming the importance of chemical composition in determining biological activity. Eucalyptus EO has likewise demonstrated strong antifungal activity. Chibi and Hassaine [45] reported significant inhibition of *Fusarium graminearum*, *Penicillium* spp., *A. flavus*, and *Trichoderma viride*, confirming its broad-spectrum antifungal potential. Beyond antifungal properties, several EOs have demonstrated antibacterial activity. Tagnaout et al. [46] reported that thyme EO showed strong antibacterial effects against *Klebsiella oxytoca*, *Serratia fonticola*, *Escherichia coli*, *Enterobacter aerogenes*, and *Enterococcus faecalis* and exhibited antifungal activity against *Candida* strains. Plant EOs have also shown higher antimicrobial activity in vapor phase than in solid phase, which supports their potential use in food preservation systems. As suggested by Severino et al. [47], EOs may be incorporated into edible films, such as methylcellulose, chitosan, and alginate coatings for fruits and vegetables. An important finding of the present study was the differential antifungal activity of the tested EOs between the contact and fumigation assays. This difference can be explained by the physicochemical properties of the volatile constituents composing each EO. In the fumigation assay, antifungal activity depends primarily on the volatility, vapor pressure, and diffusion capacity of bioactive compounds through the sealed

atmosphere of the Petri dishes. Consequently, EOs containing highly volatile monoterpenes, such as 1,8-cineole,  $\alpha$ -pinene, and p-cymene, can readily diffuse and interact with fungal hyphae without direct contact, resulting in efficient inhibition of mycelial growth. By contrast, the contact assay requires direct interaction between the fungal colony and the active compounds diffusing through the agar medium. Under these conditions, less volatile but highly lipophilic constituents, particularly phenolic monoterpenes (thymol and carvacrol) and phenylpropanoid cinnamaldehyde, may penetrate fungal cell membranes more efficiently, causing membrane disorganization, increased permeability, leakage of intracellular components, inhibition of ergosterol biosynthesis, mitochondrial dysfunction, and oxidative stress, ultimately leading to fungal growth inhibition. Furthermore, the antifungal activity of EOs is not solely determined by their major constituents but also by synergistic interactions among minor compounds, which may enhance membrane permeability and facilitate the intracellular transport of active molecules. These complementary mechanisms likely explain why cinnamon and thyme EOs consistently exhibited the highest antifungal efficacy in both bioassays, whereas EOs rich in highly volatile monoterpenes, such as eucalyptus oil, tended to perform relatively better in the fumigation assay. Collectively, these findings demonstrate that both the chemical composition of EOs and their mode of application determine their antifungal performance against *Zymoseptoria tritici*. Nevertheless, the efficacy of EOs is influenced by multiple factors, including their chemical composition and the application method. As highlighted by Chavan and Tupe [48], such compounds as carvacrol, thymol, and cinnamaldehyde exhibit potent antifungal activity against a wide range of fungal pathogens, although their efficacy may vary among fungal species owing to differences in susceptibility. Therefore, a deeper understanding of the molecular mechanisms underlying their antifungal activity, together with the development of stable and effective formulations, will be essential for maximizing their practical application in the sustainable management of *Zymoseptoria tritici* and other economically important phytopathogenic fungi. Overall, the convergence of our results with numerous previous studies confirms the broad-spectrum antifungal potential of cinnamon and thyme EOs. Their bioactivity is primarily associated with oxygenated monoterpenes and phenylpropanoids that disrupt fungal cellular structures. Nevertheless, variations in chemical composition influenced by plant origin, chemotype, environmental conditions, and extraction method may significantly affect efficacy. Future research should focus on optimizing formulation strategies and evaluating field applications to enhance their integration into sustainable wheat disease management programs. The conservation of genetic resources is essential, as it provides breeders with valuable tools to address various biotic and abiotic stresses, including the impacts of climate change and its influence on pathogen variability. As noted by Galetti Jr. [49], genetic diversity is crucial for populations to evolve in response to environmental changes, such as climate change and the emergence of new or altered diseases.

## 5. Conclusions

In Tunisia, many cultivated durum wheat varieties that were considered tolerant to STB for many years have lost their effectiveness. As a result, many farmers have resorted to the use of chemical treatments despite their negative consequences on both productivity and seed quality. Therefore, the search for new sources of tolerance among old genotypes has become urgently necessary to limit the impact of this fungus on durum wheat production in Tunisia. The results of the current investigation were encouraging, revealing a new source of tolerance among old varieties that had previously been abandoned due to their lower yield potential. To control this pathogen, incorporating the three identified tolerant varieties into future breeding programs is crucial. However,

breeders must consider several key factors to ensure the stability of newly developed varieties. These factors include the complex inheritance of fungal tolerance, as well as the high variability and evolutionary capacity of fungal populations. Controlling biotic stresses, such as STB, in wheat is particularly complex due to the rapid evolution and genetic diversity of the pathogen, which partly explains the breakdown of resistance in previously resistant genotypes. The complexity of inheritance of the control and the diversity of this fungal pathogen requires a well-planned and strategic approach to grant durable resistance. Maintaining biodiversity is of great importance, as any genotype can prove useful. Therefore, it is recommended to evaluate these tolerant varieties in different regions to assess their stability against other STB populations. The development of varieties with multiple resistance genes can help mitigate the effects of pathogen variability between populations. For these reasons, it is important to consider new sources of resistance to STB in any future durum wheat breeding program. In the other hand the presence of a pertinent antifungal activity among the tested oils can help agricultural to decrease the impact of this pathogen in durum wheat without any secondary effect.

### **Author contributions**

Fethi Bnejdi: Conceptualization, methodology, formal analysis, writing–original draft; Mourad Saadoun: Methodology, investigation; Abdelhamid Hajji: Methodology, data curation; Iness Jlassi: Methodology, resources; Teyssir Bouattour: Formal analysis, writing–original draft; Anis Ben Hsouna: Investigation, writing–review & editing, visualization; Miroslava Kačániová: Formal analysis, investigation, writing–review & editing, visualization; Sonia Marghali: Software, data curation; Mossadok Ben-Attia: Formal analysis, investigation, writing–review & editing; Wissem Mnif: Formal analysis, writing–review & editing, visualization, supervision; Angelo Maria Giuffrè: Software, writing–review & editing, visualization, supervision, project administration; Safia El-Bok: Conceptualization, methodology, formal analysis, investigation, resources, data curation, writing–original draft. All authors have read and approved the final version of the manuscript for publication.

### **Use of AI tools declaration**

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

### **Acknowledgments**

The authors are thankful to the Deanship of Graduate Studies and Scientific Research at University of Bisha for supporting this work through the Fast-Track Research Support Program.

### **Conflict of interest**

Angelo Maria Giuffrè is the Guest Editor of special issue "The extraction and application of essential oils" for AIMS Agriculture and Food. Angelo Maria Giuffrè was not involved in the editorial review and the decision to publish this article.

## References

1. Shewry PR, Hey SJ (2015) The contribution of wheat to human diet and health. *Food Energy Secur* 4: 178–202. <https://doi.org/10.1002/fes3.64>
2. Mekuria BM, Zewudie T, Mamo D (2025) Prevalence and impact of Septoria tritici blotch (Zymoseptoria tritici) on bread wheat production in Ethiopia. *Discover Plants* 2: 343. <https://doi.org/10.1007/s44372-025-00435-6>
3. Fones H, Gurr S (2015) The impact of *Septoria tritici blotch* disease on wheat: An EU perspective. *Fungal Genet Biol* 79: 3–7. <https://doi.org/10.1016/j.fgb.2015.04.004>
4. Torriani SFF, Melichar JPE, Mills C, et al. (2015) *Zymoseptoria tritici*: A major threat to wheat production. *Mol Plant Pathol* 16: 381–397.
5. Ben M'Barek S, Karisto P, Abdedayem W, et al. (2023) Improved control of *Septoria tritici blotch* in durum wheat using cultivar mixtures. *Agronomy* 13: 2638. <https://doi.org/10.3390/agronomy13102638>
6. McDonald BA, Linde C (2002) Pathogen population genetics, evolutionary potential, and durable resistance. *Annu Rev Phytopathol* 40: 349–379. <https://doi.org/10.1146/annurev.phyto.40.120501.101443>
7. Torriani SFF, Melichar JPE, Mills C, et al. (2015) *Zymoseptoria tritici*: A major threat to wheat production, integrated approaches to control. *Mol Plant Pathol* 16: 381–397. <https://doi.org/10.1016/j.fgb.2015.04.010>
8. Porras R, Miguel-Rojas C, Lorite IJ, et al. (2023) Characterization of durum wheat resistance against *Septoria tritici blotch* under climate change conditions of increasing temperature and CO<sub>2</sub> concentration. *Agronomy* 13: 2638. <https://doi.org/10.3390/agronomy13102638>
9. Hassine M, Bnejdi F, Bahri BA, et al. (2022) Detection of maternal and cytoplasmic effects on resistance to *Zymoseptoria tritici* in durum wheat. *BioMed Res Int* 2022: 8497417. <https://doi.org/10.1155/2022/8497417>
10. Velasquez AC, Castroverde CDM, He SY (2018) Plant–pathogen warfare under changing climate conditions. *Curr Biol* 28: R619–R634. <https://doi.org/10.1016/j.cub.2018.03.054>
11. Ponomarenko A, Goodwin SB, Kema GHJ (2019) *Septoria tritici blotch* (STB) of wheat: Biology, epidemiology, and control. *Annu Rev Phytopathol* 57: 203–227.
12. Hitchcock M, Xu J (2026) Roles of mutation, ploidy, and recombination in adaptive evolution in two divergent model yeasts. *Genes (Basel)* 17: 204. <https://doi.org/10.3390/genes17020204>
13. Bnejdi F, Saadoun M, El Gazzah M (2011) Genetic adaptability of the inheritance of resistance to different levels of aggressiveness of *Septoria tritici* isolates in durum wheat. *Crop Prot* 30: 1280–1284. <https://doi.org/10.1016/j.cropro.2011.03.013>
14. Aktar W, Sengupta D, Chowdhury A (2009) Impact of pesticide use in agriculture: Their benefits and hazards. *Interdiscip Toxicol* 2: 1–12. <https://doi.org/10.2478/v10102-009-0001-7>
15. Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Res* 14: 415–421. <https://doi.org/10.1111/j.1365-3180.1974.tb01084.x>
16. Saari EE, Prescott JM (1975) A scale for appraising the foliar intensity of wheat diseases. *Plant Dis Rep* 59: 377–380.
17. Eyal Z, Scharen AL, Prescott JM, et al. (1987) *The Septoria diseases of wheat: Concepts and methods of disease management*, CIMMYT, Mexico.
18. Castro AC, Simón MR (2016) Effect of tolerance to *Septoria tritici blotch* on grain yield, yield components and grain quality in Argentinean wheat cultivars. *Crop Prot* 90: 66–76. <https://doi.org/10.1016/j.cropro.2016.08.015>

19. Chartrain L, Brading PA, Widdowson JP, et al. (2004) Partial resistance to septoria tritici blotch in the wheat cultivars Arina and Riband. *Phytopathology* 94: 497–504. <https://doi.org/10.1094/PHYTO.2004.94.5.497>
20. Ben M'Barek K, da Silva JAT (2011) Effects of Septoria leaf blotch infection on grain yield and its components of three bread wheat genotypes (*Triticum aestivum* L.). *Fruit Veg Cereal Sci Biotechnol* 5: 80–85.
21. Dreisigacker S, Sehgal D, Jaimez AR, et al. (2015) CIMMYT wheat molecular genetics: Laboratory protocols and applications. CIMMYT, Mexico.
22. Farzaneh M, Ahmadzadeh M, Hadian J, et al. (2015) Chemical composition and antifungal activity of essential oils against plant pathogenic fungi. *J Agr Sci Tech* 17: 1245–1256.
23. Lynch M, Walsh B (1998) *Genetics and analysis of quantitative traits*, Sunderland, MA: Sinauer Associate.
24. Binalf L, Shifa H, Tadesse W (2024) Association mapping of Septoria tritici blotch resistance in bread wheat in Bale and Arsi highlands, Ethiopia. *Heliyon* 10: e32265. <https://doi.org/10.1016/j.heliyon.2024.e32265>
25. Linde CC, Zhan J, McDonald BA (2002) Population structure of *Mycosphaerella graminicola*: From lesions to continents. *Phytopathology* 92: 946–955. <https://doi.org/10.1094/PHYTO.2002.92.9.946>
26. Linde CC, Zhan J, McDonald BA (2002) Population structure of the plant pathogenic fungus *Mycosphaerella graminicola* (anamorph *Zymoseptoria tritici*) in a single field in Switzerland. *Phytopathology* 92: 1137–1144. <https://doi.org/10.1094/PHYTO.2002.92.10.1137>
27. Alouane T, Rimbert H, Bormann J, et al. (2021) QTL mapping in *Fusarium graminearum* identified an allele of FgVe1 involved in reduced aggressiveness. *Int J Mol Sci* 22: 6257. <https://doi.org/10.3390/ijms22126257>
28. Kassie MM, Abebe TD, Desta EA, et al. (2025) Multi-environment evaluation of bread wheat genotypes for yield stability in Ethiopia using AMMI and GGE-biplot analyses. *Crop Breeding Gene Genomics* 7: e250004. <https://doi.org/10.20900/cbpg20250004>
29. Bellameche F, Pedrazzini C, Mauch-Mani B, et al. (2020) Efficiency of biological and chemical inducers for controlling Septoria tritici leaf blotch (STB) on wheat (*Triticum aestivum* L.). *Eur J Plant Pathol* 158: 99–109. <https://doi.org/10.1007/s10658-020-02057-y>
30. Allagui MB, Moumni M, Romanazzi G (2024) Antifungal activity of thirty essential oils to control pathogenic fungi of postharvest decay. *Antibiotics* 1: 13–28 <https://doi.org/10.3390/antibiotics13010028>
31. Zheng S, Jing G, Wang X, et al. (2019) Antifungal activity of essential oils against *Villosiclava virens*, the causal agent of rice false smut. *Ind Crop Prod* 128: 206–211.
32. Ranasinghe P, Pigera S, Premakumara GAS, et al. (2013) Medicinal properties of 'true' cinnamon (*Cinnamomum zeylanicum*): A systematic review. *BMC Complem Altern M* 13: 275. <https://doi.org/10.1186/1472-6882-13-275>
33. Denkova-Kostova R, Teneva D, Tomova T, et al. (2021) Mechanisms of action of cinnamaldehyde against fungal pathogens. *Molecules* 26: 6157. <https://doi.org/10.3390/molecules26206157>
34. Tabti L, Dib MEA, Djabou N, et al. (2014) Control of fungal pathogens of *Citrus sinensis* L. by essential oil and hydrosol of *Thymus capitatus* L. *J Appl Bot Food Qual* 87: 279–285.
35. Wei Y, Bi Y, Xue H, et al. (2020) Antifungal activity of cinnamaldehyde against *Fusarium sambucinum* involves inhibition of ergosterol biosynthesis. *J Appl Microbiol* 129: 256–265. <https://doi.org/10.1111/jam.14601>

36. Liu W, Wang T, Su E (2024) Insights into the antifungal activity and mechanisms of cinnamon components against *Aspergillus flavus* and *Penicillium citrinum*. *Food Res Int* 197:115291. <https://doi.org/10.1016/j.foodres.2024.115291>
37. Omidbeygi M, Barzegar M, Hamidi Z, et al. (2007) Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. *Food Control* 18: 1518–1523. <https://doi.org/10.1016/j.foodcont.2006.12.003>
38. Benoutman A, Erbiai EH, Edderdaki FZ, et al. (2022) Phytochemical composition, antioxidant and antifungal activity of *Thymus capitatus* collected from northern Morocco. *Antibiotics* 11: 681. <https://doi.org/10.3390/antibiotics11050681>
39. Pinto E, Vale-Silva L, Cavaleiro C, et al. (2006) Antifungal activity of essential oils of *Thymus* species. *J Med Microbiol* 55: 1367–1373. <https://doi.org/10.1099/jmm.0.46443-0>
40. Pinto E, Pina-Vaz C, Salgueiro L, et al. (2009) Antifungal activity of the essential oil of *Thymus* species against *Candida*, *Cryptococcus*, and *Aspergillus* species. *J Med Microbiol* 58: 1454–1462. <https://doi.org/10.1099/jmm.0.010538-0>
41. Chavan PS, Tupe SG (2014) Antifungal activity and mechanism of action of carvacrol and thymol against fungal pathogens. *J Appl Microbiol* 117: 1250–1260.
42. Socović M, Glamočlija J, Marin PD, et al. (2019) Antifungal activity of essential oils rich in thymol against fungal pathogens. *J Appl Microbiol* 126: 780–789.
43. Ben Jabeur M, Hamada (2015) Antifungal activity of *Thymus capitatus* essential oil chemotypes against fungal pathogens: Influence of phenolic compounds. *Ind Crop Prod* 76: 532–538.
44. Maissa BJ, Walid H (2015) Antifungal activity of chemically different essential oils from wild Tunisian *Thymus* spp. *Nat Prod Res* 29: 869–873. <https://doi.org/10.1080/14786419.2014.984182>
45. Chibi F, Hassaine H (2023) Antifungal activity of eucalyptus essential oils against cereal pathogenic fungi. *J Appl Res Med Aroma* 32: 100450.
46. Tagnaout I, Zerkani H, Hadi N, et al. (2022) Chemical composition, antioxidant and antibacterial activities of *Thymus capitatus* (L.) Hoffmann and Link essential oils. *Plants (Basel)* 11: 954. <https://doi.org/10.3390/plants11070954>
47. Severino R, Vu KD, Lacroix M (2014) Incorporation of essential oils into edible coatings for food preservation. *Food Res Int* 64: 504–513.
48. Chavan PS, Tupe SG (2014) Antifungal activity and mechanism of action of carvacrol and thymol against vineyard and wine spoilage yeasts. *Food Control* 46: 115–120.
49. Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. *Nature* 470: 479–485. <https://doi.org/10.1038/nature09670>



AIMS Press

© 2026 the Author(s), licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0>)