Chemical Composition of Essential Oils and Their Antifungal Activity in Controlling Ascochyta rabiei

A. Ennouri¹, ²*, A. Lamiri¹, M. Essahli¹, and S. Krimi Bencheqroun²

ABSTRACT

Investigation for developing natural plant protection products as an alternative to synthetic fungicides has become important regarding the environmental impact. In this study, the chemical composition and the antifungal activity of five Moroccan aromatic plants, namely, oregano (Origanum compactum), thyme (Thymus vulgaris), Eucalyptus (Eucalyptus camaldulensis), mint (Mentha pulegium L) and myrtle (Myrtus communis) was explored for controlling Ascochyta rabiei, in vitro. The pathogen is a seed-borne causal agent of Ascochyta blight and it is considered the most economically damaging disease of chickpea. The radial growth of A. rabiei was completely inhibited by oregano, mint, thyme and myrtle at low concentrations (0.15-5 µL mL⁻¹). The most important effect was obtained with oregano (Minimum Inhibitory Concentration, MIC- 0.15 µL mL⁻¹), followed by Thyme (MIC- 0.5 µL mL⁻¹). The phytotoxicity test of these essential oils on chickpea germination showed that oregano and Thyme oils do not have phytotoxic effects at MIC concentrations, whereas mint and myrtle oils can have an effect on reducing germination percentage of chickpea seeds. The chemical composition of tested essential oils was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS analysis). The analysis revealed the dominance of two compounds (Thymol and Carvacrol) in the most effective oils and can represent the principal active ingredient in the pathogen control. Therefore, the essential oils of oregano and Thyme or their major compounds could be investigated for seed or foliar treatment of chickpea against Ascochyta blight infection.

Keywords: Chickpea, GC-MS analysis, Oregano, Thyme.

INTRODUCTION

Ascochyta blight caused by Ascochyta rabiei (Pass.) Lab. is one of the greatest biotic stresses reducing potential yield in chickpea over the world (Akamatsu et al., 2012). In Morocco, the disease is considered the most important foliar disease during several years of surveys (Krími Bencheqroun et al., 2014; Saxena et al., 1996). Yield losses can reach 97% during some epidemic years (Baite and Bubey, 2018). The pathogen is a seed-borne causal agent of Ascochyta blight. It can infect all aboveground parts of the plant and attacks the crop at both vegetative and pudding stages.

Synthetic fungicides are usually used for disease control as seed treatment or foliar applications. Seed treatments are applied on chickpea to protect the emerging seedling in the field from early infection of Ascochyta blight. Although the synthetic fungicides are known to be effective, their permanent or repeated application can induce the development of resistance to several fungicides. They can also cause many environmental problems and toxicity to non-protected organisms. (Lima et al., 2008).

Investigation for developing natural plant protection product as an alternative to synthetic fungicides becomes important regarding the environmental impact.

The benefit of essential oils is that they are
naturally biodegradable, non-pollutants, and they are active in vapor phase (Serrano et al., 2005; Sharma et al., 2008). Fungicidal activities of many plants against several pests have been confirmed (Boulenouar et al., 2014; Abdelgaleil et al., 2019; Amini and Bahramian, 2019). A number of studies reported the antifungal activities of essential oils products but few of them have confirmed their antifungal properties against seed borne fungi on legumes (Enzo, M., et al., 2012).

Morocco is rich of native species of aromatique and medicinal plants. Oregano (Origanum compactum), Thyme (Thymus vulgaris), Eucalyptus (Eucalyptus camaldulensis), mint (Mentha pulegium L.) and myrtle (Myrtus communis) are widely present naturally or cultivated in Morocco since antiquity and are known for their medicinal and aromatic properties.

The chemical composition of essential oils may vary depending on plant genotype, growing conditions, and extraction method (Hammer et al., 1999). Therefore, chemical analysis using Gas Chromatography–Mass Spectrometry (GC-MS) is necessary in parallel with pathogenic activity tests and can help for comparison to other essential oils of known antifungal activity.

The objectives of this research were: (1) To evaluate the efficacy of some essential oils to control a seed borne pathogen A. rabiei of chickpea and determine the Minimum Inhibitory Concentration of essential oil needed to control pathogen growth (MIC), (2) To determine if the essential oils are phytotoxic on chickpea seed germination, and (3) To analyze the essential oils composition by using GC-MS.

## MATERIALS AND METHODS

### Plant Materials and Essential Oil Extraction

The following medicinal plants: Oregano (O. compactum), Thyme (T. vulgaris), Eucalyptus (E. camaldulensis), mint (M. pulegium L.) and myrtle (M. communis) were harvested in their natural habitat in different locations in Morocco (Table 1). The plants were identified in Laboratory of Applied Chemistry and Environment, Faculty of Science and Techniques, Settat, Morocco.

The essential oils of these plants were extracted by steam distillation in a Clevenger’s apparatus for 3 hours, following the method of Guenther (1948). The essential oils were then kept at 4°C until use.

### Antifungal Activity Assays in Vitro

The pathogen A. rabiei was isolated from infested plants of chickpea collected from the locality of 32° 21’ N, 8° 51’ W and 175m above the sea level in Morocco. Fungi was multiplied on Chickpea Meal Agar medium (CPMA) and incubated at 22°C (12 hours photoperiod) for 7 days.

The assay of antifungal activity was performed on solid CPMA medium amended with different oils at the following concentrations: 0% (control), 0.0025, 0.005, 0.01, 0.015, 0.025,0.05, 0.15, 0.5 and 1%. The essential oils were prepared by dissolving them in Tween 20 (0.5%, v/v) and added to CPMA immediately before pouring into 90 mm Petri dishes. A. rabiei

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### Table 1. List of essential oils used in this study and their origins.

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Plant origin</th>
<th>Family</th>
<th>Origin regions</th>
<th>Local name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregano</td>
<td><em>Origanum compactum</em></td>
<td>Lamiaceae</td>
<td>Ouazzane</td>
<td>Z’itrah</td>
</tr>
<tr>
<td>Thyme</td>
<td><em>Thymus vulgaris</em></td>
<td>Lamiaceae</td>
<td>Marrakech</td>
<td>Zaâtar</td>
</tr>
<tr>
<td>Mint</td>
<td><em>Mentha pulegium</em> L.</td>
<td>Lamiaceae</td>
<td>Marrakech</td>
<td>Flio</td>
</tr>
<tr>
<td>Myrtle</td>
<td><em>Myrtus communis</em></td>
<td>Myrtaceae</td>
<td>Marrakech</td>
<td>Rihan</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td><em>Eucalyptus camaldulensis</em></td>
<td>Myrtaceae</td>
<td>Khouribga</td>
<td>Calibtus</td>
</tr>
</tbody>
</table>
was inoculated immediately by plating in the center 5 mm plugs from actively growing cultures. The Petri dishes were incubated at 22°C and 12 hours of photoperiod.

Radial growth of colonies was evaluated every 2 days until day 21.

Percentage of Inhibition of Mycelial Growth (MGI) by oils was calculated after 21 days using the following equation:

\[ \text{MGI} \left( \% \right) = \left( \frac{dc - dt}{dc} \right) \times 100 \]

Where, dc and dt represent mycelia growth diameter in control and treatment, respectively.

The Minimum Inhibitory Concentration (MIC) that produced 100% growth reduction was estimated for each compound 21 days after inoculation.

At the end of the incubation period, the nature of essential oils (fungistatic or fungicidal) was determined by the Thompson method (Thompson, 1989). The agar discs of the fungi, which did not show any visible growth were transferred to essential oils-free CPMA plates and incubated for further 10 days to observe the revival growth. The actions of essential oils were fungicidal if the pathogen was not growing, or fungistatic if the pathogen growth occurred.

**Phytotoxicity of the Essential Oils**

The standard germination test was used to determine phytotoxicity of the oils according to AOSA (1990). The essential oils were applied to the seeds at three different rates: MIC, MIC\(\times\)2, and MIC\(\times\)5, mixed with 5 mL distilled water and 0.5 mL ethanol.

The seeds were disinfected with a 5% sodium hypochlorite solution for 2 minutes, then cleaned twice with sterilized water and mixed with oils. Four replicates of fifty seeds were then transferred to filter paper in Petri dishes, and incubated at 21°C for 10 days. During the experiment, water was added as necessary.

Germination was defined by counting the germinated seeds every day for up to 10 days. Seeds were classified as germinated when the length of the radicals exceeded 2 mm. The Mean Germination Time (MGT) was determined using the equations of Ellis and Roberts (1981):

\[ MGT = \frac{\sum Di \cdot Ni}{N} \]

Where, N, the Number of seeds germinated on the day i, D, the Days of germination test, N the total Number of seeds.

The Germination Index (GI) was computed using the following formula according to AOSA (1983):

\[ G = \frac{\text{No of germinated seeds}}{\text{day of first count}} + \ldots + \frac{\text{No of germinated seeds}}{\text{day of final count}} \]

Seedling Vigor Index (SVI) was determined by Abdul-Baki and Anderson (1973) as follows:

\[ \text{SVI} = \text{Germination} \left( \% \right) \times \text{Radical length} \ (\text{cm}) \]

**Identification of the Chemical Composition of Essential Oils**

The chemical composition of the essential oils was analyzed using Gas Chromatography Mass Spectrometry (GC/MS). The essential oils were volumetrically diluted a million times in ethyl acetate before injection in the Gas Chromatography (GC).

Agilent Technologies 7890A Gas Chromatograph was used to analyze the chemical composition of the essential oils. It is equipped with Mass Selective Detector (MSD) and an HP-5MS capillary column 30 m long and 0.25 mm diameter. The carrier gas was helium with a flow rate of 1 mL/min. The initial temperature of the column was 50°C, increased to 150°C (with 3°C min\(^{-1}\)), and maintained at 250°C (with 10°C min\(^{-1}\)). A volume of 1 μL of every sample was injected in split mode. The mass percentage of the different constituents of essential oils is given in relative peak area. The fragmentation was carried out in a 70eV electric field.
Statistical analyses

Data were subjected to Analysis Of Variance (ANOVA) using GenStat Procedure Library Release PL23.1. The significance of differences among treated samples was evaluated using Duncan’s multiple range tests.

RESULTS AND DISCUSSION

Antifungal Activity of Essential oils in Vitro

The effects of essential oils on mycelial growth of A. rabiei are shown in Figure 1. Growth of the pathogen’s mycelium was observed during the first 72 hours of incubation. The essential oils tested (oregano, thyme, mint, and myrtle) had a significant activity against fungal growth, with the concentrations of 0.015, 0.025, 0.05, and 0.15%.

However, in the case of Eucalyptus oil, there is no significant antifungal activity compared to the control (Figure 1-e).

The Minimum Inhibitory Concentration (MIC) was determined for all essential oils, (Figure 2). Four essential oils completely suppressed pathogen growth (MGI= 100%) within the 21 days period of the experiments. These oils were oregano,
Composition and Antifungal Activity

Oregano controlled pathogen growth at a smallest concentration of 0.15 μL mL⁻¹ (Figure 2), followed by Thyme oil at a concentration of 0.5 μL mL⁻¹. Soto-Mondivil et al., (2006) demonstrated also an important antifungal activity of the essential oil of T. vulgaris against Alternaria citri. Other research shows that the pathogen A. rabiei has been inhibited by other oils such as Salvia officinalis and Salvia tomentosa oils (Yilar and Bayar, 2019). The essential oil of M. pulegium L has also a significant inhibitory activity towards A.rabiei. The pathogen was completely inhibited at a minimum concentration of 1.5 μL mL⁻¹. Bayar (2018) proved that Menthas picata L essential oil has a strong antifungal activity against different isolates of A.rabiei in vitro conditions at 10 μL mL⁻¹. Myrtle exhibited an antifungal action with a concentration of 5 μL mL⁻¹. Further researches proved that Myrtle oil has bioactive properties, especially antifungal activity to Fusarium sp., Drechslera sp. and Macrophomina phaseolina (Starović et al., 2016). In the same way, Bayan et al., (2017) confirmed that the essential oil of Myrtus communis L inhibits 58% of mycelial growth of three different isolates of Ascochyta blight pathogen with a concentration of 8 μL mL⁻¹.

However, the Eucalyptus oil had a small antifungal effect in our study and allowed only 30% of inhibition rate at dose of 10 μL mL⁻¹.

Two studies performed with the essential oil of Eucalyptus reported that a concentration higher than 2.5 μL mL⁻¹ was required for observing growth inhibition against C. gloeosporioides (Combrinck et al., 2011; Padman and Janardhana, 2012). Therefore, a concentration higher than 10 μL mL⁻¹ of essential oil of Eucalyptus may be required to observe minimal mycelial growth inhibition of A. rabiei.

To determine the antifungal characteristics of essential oils, the inhibited mycelium was transferred to new media without oils. In all cases, pathogen mycelium was not able to grow again in the new media except of the inhibited mycelium with Myrtle oil (Table 2). Therefore, the majority of tested oils (oregano, thyme, and mint) could have a fungicidal property at MIC concentrations.
Table 2. Property of antifungal activities of essential oils on growth inhibition of *A. rabiei* in new media of CPMA.

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Concentration (μL mL⁻¹)</th>
<th>Mycelial growth diameter (cm) in the new media</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. compactum</em></td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td><em>T. vulgaris</em></td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>M. pulegium</em></td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>M. communis</em></td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

and could kill the pathogen. However, myrtle oil could have a fungistatic property and stop the mycelium growth only.

**Phytotoxicity of Essential Oils to Chickpea Seeds**

The phytotoxicity effect of four essential oils (oregano, thyme, mint and myrtle) on chickpea germination was evaluated using three concentrations (MIC, MIC×2, and MIC×5). The results indicated that by enhancing the concentrations of essential oils, germination percentage were significantly (P≤ 0.05) decreased (Table 3). Essential oils of oregano and Thyme oils do not have an effect on germination percentage in comparison to the control at concentrations up to MIC×2, but they can affect germination at the greatest concentration (MIC×5). Delayed germination and less GI with essential oil of oregano and Thyme at MIC×5 concentration may reflect the presence of allelochemicals in maximum concentration. Shanee *et al.* (2011) reported similar results by using extract of different parts of *Euphorbia dracunculoides*, which caused maximum reduction in germination, MGT, and GI of chickpea, whereas, mint and myrtle oils can completely inhibit the seed germination of chickpea at low concentrations (MIC). Umran and Bapeer (2011) showed that the volatile substances released from the Myrtle leaves have a significant effect on the germination of the chickpea seeds.

Chickpea seed Germination Index (GI) and Seedling Vigor Index (SVI) of chickpea were also not affected by using oregano and Thyme oils at MIC concentrations (Table 3). Therefore, these essential oils did not have phytotoxic effects at low concentrations. However, mint and myrtle oils can be phytotoxic on chickpea seeds germination.

Chemical Compositions of Essential OilsThe most important compounds of five essential oils oregano, thyme, mint and myrtle and *Eucalyptus* and their percentage was obtained using GC/MS (Table 4). The predominant constituents of oregano were carvacrol (38.67%), thymol (25.90%), and γ-terpinene (17.56%), which constituted approximately 82% of the oil. Several studies reported the same chemical composition present in the oregano oil samples, which is dominated by carvacrol and thymol (Den Broucke and Lemli, 1980; Bouhdid *et al.*, 2008). Martínez Romero *et al.* (2007) proved that carvacrol was useful in limiting the growth of *Botrytis cinerea* on grape berries and thus preventing fruit rot.

In thyme oil, 24 compounds were identified with the dominance of 3 compounds; thymol (41.39%), γ-terpinene (22.25%), and p-cymen (15.59%). Other researches confirm the dominance of the same compound thymol (Sajjadi and...
### Table 3. Phytotoxic effect of essential oils on germination of chickpea seeds.

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Concentration (µL L⁻¹)</th>
<th>G (%)</th>
<th>GI</th>
<th>MGT</th>
<th>SVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>100²</td>
<td>4.74⁴</td>
<td>5.78⁴</td>
<td>618.1⁴</td>
</tr>
<tr>
<td>Oregano</td>
<td>15 (MIC)</td>
<td>99⁴</td>
<td>5.01⁴</td>
<td>4.81⁴</td>
<td>561.8⁴</td>
</tr>
<tr>
<td></td>
<td>30 (MIC×2)</td>
<td>88⁴</td>
<td>4.41⁴</td>
<td>4.39⁴</td>
<td>178.3⁴</td>
</tr>
<tr>
<td></td>
<td>150 (MIC×5)</td>
<td>66⁴</td>
<td>3.53⁴</td>
<td>3.25⁴</td>
<td>61.5⁴</td>
</tr>
<tr>
<td>Thyme</td>
<td>50 (MIC)</td>
<td>96⁴</td>
<td>5.15⁴</td>
<td>4.52⁴</td>
<td>304.7⁴</td>
</tr>
<tr>
<td></td>
<td>100 (MIC×2)</td>
<td>71⁴</td>
<td>3.86⁴</td>
<td>3.37⁴</td>
<td>77.8⁴</td>
</tr>
<tr>
<td></td>
<td>500 (MIC×5)</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
</tr>
<tr>
<td>Mint</td>
<td>150 (MIC)</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
</tr>
<tr>
<td></td>
<td>300 (MIC×2)</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
</tr>
<tr>
<td></td>
<td>1500 (MIC×5)</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
</tr>
<tr>
<td>Myrtle</td>
<td>500 (MIC)</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
</tr>
<tr>
<td></td>
<td>1000 (MIC×2)</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
</tr>
<tr>
<td></td>
<td>5000 (MIC×5)</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
<td>0⁴</td>
</tr>
</tbody>
</table>

² The letters Data followed with the same number are not significantly different (P= 0.05).

### Table 4. Chemical composition (%) of essential oils of oregano, thyme, mint, myrtle, and Eucalyptus from Morocco by gas chromatography/mass spectroscopy (GC/MS).

<table>
<thead>
<tr>
<th>Compound a</th>
<th>RT b</th>
<th>RI c</th>
<th>Oregano</th>
<th>Thyme</th>
<th>Mint</th>
<th>Myrtle</th>
<th>Eucalyptus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% peak area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anisole</td>
<td>3.10</td>
<td>923</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.31</td>
</tr>
<tr>
<td>α-thujene</td>
<td>3.28</td>
<td>925</td>
<td>1.76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tricyclene</td>
<td>3.41</td>
<td>926</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.14</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>3.58</td>
<td>931</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13.22</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>6.58</td>
<td>974</td>
<td>1.63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α-Terpinene</td>
<td>8.50</td>
<td>1015</td>
<td>3.25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-Cymen</td>
<td>9.96</td>
<td>1023</td>
<td>15.59</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.56</td>
</tr>
<tr>
<td>Limonene</td>
<td>10.13</td>
<td>1027</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.51</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>11.10</td>
<td>1036</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>48.81</td>
</tr>
<tr>
<td>γ-Terpinène</td>
<td>11.34</td>
<td>1057</td>
<td>17.56</td>
<td>22.25</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linalol</td>
<td>12.94</td>
<td>1100</td>
<td>1.79</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4-Terpinol</td>
<td>15.85</td>
<td>1176</td>
<td>2.15</td>
<td>1.15</td>
<td>-</td>
<td>-</td>
<td>3.84</td>
</tr>
<tr>
<td>Myrtenal</td>
<td>16.79</td>
<td>1197</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11.34</td>
</tr>
<tr>
<td>Nopol</td>
<td>16.30</td>
<td>1212</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.66</td>
</tr>
<tr>
<td>Careen (2)</td>
<td>17.50</td>
<td>1227</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.01</td>
</tr>
<tr>
<td>Carvone</td>
<td>18.82</td>
<td>1231</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.81</td>
</tr>
<tr>
<td>Thymolmylether</td>
<td>19.03</td>
<td>1233</td>
<td>1.18</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carvaryl methyl oxide</td>
<td>19.96</td>
<td>1244</td>
<td>5.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Geraniol</td>
<td>20.40</td>
<td>1245</td>
<td>-</td>
<td>-</td>
<td>14.72</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pulegone</td>
<td>20.42</td>
<td>1247</td>
<td>-</td>
<td>-</td>
<td>84.75</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thymol</td>
<td>21.50</td>
<td>1293</td>
<td>25.9</td>
<td>41.39</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>21.87</td>
<td>1311</td>
<td>38.67</td>
<td>2.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Myrtenyl acetate</td>
<td>23.20</td>
<td>1328</td>
<td>-</td>
<td>-</td>
<td>36.67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>26.76</td>
<td>1417</td>
<td>1.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Guaiol</td>
<td>42.54</td>
<td>1596</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.28</td>
</tr>
<tr>
<td>Cedrol</td>
<td>42.52</td>
<td>1607</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16.13</td>
</tr>
</tbody>
</table>

² The compounds that present lower than 1% are not shown.  

b Retention Time (Min).  
c Retention Indices on the HP 5MS column.
Thymol and carvacrol demonstrated antifungal activity against vine and wine yeasts in vitro and in vivo conditions (Chavan and Tupe, 2014). In the essential oil extracted from mint, 8 compounds were identified, where Pulegone presented the highest percentage (84.75%) followed by geraniol (14.72%). Further research has shown that pulegone is the principal component of mint essential oil, but in varying proportions (Boukhebti et al., 2011; Ait-Ouazzou et al., 2012; Cherrat et al., 2014; Ouakouak et al., 2015). The antifungal potential of the essential oil of M. pulegium L can be attributed to its chemical composition. Indeed, this plant is dominated by kenotic molecules (pulegone and geraniol) that are more active against microbial agents thanks to the presence of the oxygen atom (Satrani, 2010; Dorman and Deans, 2000).

The major compounds of myrtle oil, were 1.8-cineole (48.81%) followed by acetate myrtenyl (36.67%) and α-pinene (13.22%). Eucalyptus oil contained also 1.8-cineole as major element (34.22%) followed by cedrol (16.13%) and myrtenal (11.34%). Various species of Eucalyptus were also characterized with high yield of 1,8 cineole like E. globules (Dellacassa et al., 1990).

In another study, the individual application of thymol and carvacrol showed a highly significant antifungal effect against the pathogens Colletotrichum acutatum and Botryodiplodia theobroma (Numpaque et al., 2011). They indicated that both compounds could be alternatives to traditional chemical fungicides for control of pre- and post-harvest fungi on fruit and vegetable species (Numpaque et al., 2011). Therefore, these compounds (thymol and carvacrol) may constitute the main active compounds against the pathogen A. rabiei. Further studies may be considered to test the efficacy of these compounds against the pathogen.

CONCLUSIONS

In this study, the efficacy of five essential oils (oregano, thyme, mint, myrtle, and Eucalyptus) against pathogen A. rabiei was evaluated in vitro. A highly significant antifungal activity of O. compactum and T. vulgaris oils was identified at low concentrations without phytotoxic effect on chickpea seeds germination. The analysis of their chemical composition showed the dominance of two compounds (thymol and carvacrol) that are absent in the other oils. Therefore, it is recommended to test these oils as seed treatment in vivo conditions and to evaluate the efficacy of the different major compounds individually. This study will initiate further research using these essential oils and/or their compounds in the formulation of biological products that may be alternatives to chemical fungicides.

ACKNOWLEDGEMENTS

This work is a part of PhD. study supported by University Hassan I and National institute of Agriculture Research (INRA) (Settat, Morocco). The author acknowledges the financial support of PhD. scholarship of the National Center for Scientific and Technical Research (CNRST).

REFERENCES


چکیده
با در نظر گرفتن اثرات قارچ کش های مصنوعی روی محیط زیست، پژوهش برای جایگزین کردن آنها با مواد طبیعی حفظ گیاه اهمیت یافته است. در این پژوهش، برای کنترل Ascochyta rabiei ترکیب شیمیایی و فعالیت ضد قارچی پنج گیاه معطر مراکش به نام های Oregano (Origanum compactum), Thyme (Thymus vulgaris), Eucalyptus (Eucalyptus camaldulensis), Mint (Mentha pulegium L.) و Myrtle (Myrtus communis L.) در شیشه آزمایشگاه مورد بررسی قرار گرفت. این بیمارگر بذر بُرد (seed-borne) عامل بهاری آسکوچیتا (Ascochyta) را در اکثریت بیماری های بذر نخود تلقی می‌کند. در این آزمایش، Oregano، Mint، Thyme و Myrtle در ضعاید محلول (0.15-5 µL/mL) رشد و گسترش A. rabiei را به طور کامل متوقف کردند. مهم ترین تأثیر از مصرف Oregano با غلظت بازدارنده کمینه (MIC) برابر 0.15 µL/mL در مسئولیت آسکوچیتا (Ascochyta) در طیف سنجی جرمی (GC-MS) بود. نتایج تجزیه و تحلیل نشان داد که در مادرکاری، مواد ماکننگی ماده غلاف اصلی در کنترل بهبود و Oregano به عنوان یک مواد ماده و聘یت آسکوچیتا مورد بررسی قرار داد.