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

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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 (Krimi 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 products as an alternative to synthetic fungicides becomes important regarding the environmental impact.

The benefit of essential oils is that they are

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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*

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

Essential oils	Plant origin	Family	Origin regions	Local name
Oregano	*Origanum compactum*	Lamiaceae	Ouazzane	Z'itrah
Thyme	*Thymus vulgaris*	Lamiaceae	Marrakech	Zaâtar
Mint	*Mentha pulegium* L	Lamiaceae	Marrakech	Flio
Myrtle	*Myrtus communis*	Myrtaceae	Marrakech	Rihan
Eucalyptus	*Eucalyptus camaldulensis*	Myrtaceae	Khouribga	Calibtus

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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:

$$\mathrm{MGI}\ (\%) = \frac{\mathrm{dc} - \mathrm{dt}}{\mathrm{dc}} \times 100$$

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×2, and MIC×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):

$$MGT = \frac{\sum Di\,.Ni}{N}$$

Where, N_i the Number of seeds germinated on the day i, D_i 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):

$$\mathrm{G} = \frac{No of germinated seeds}{days of first count} + \cdots + \frac{No of germinated seeds}{days of final count}$$

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

SVI= Germination (%)× Radical length (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,

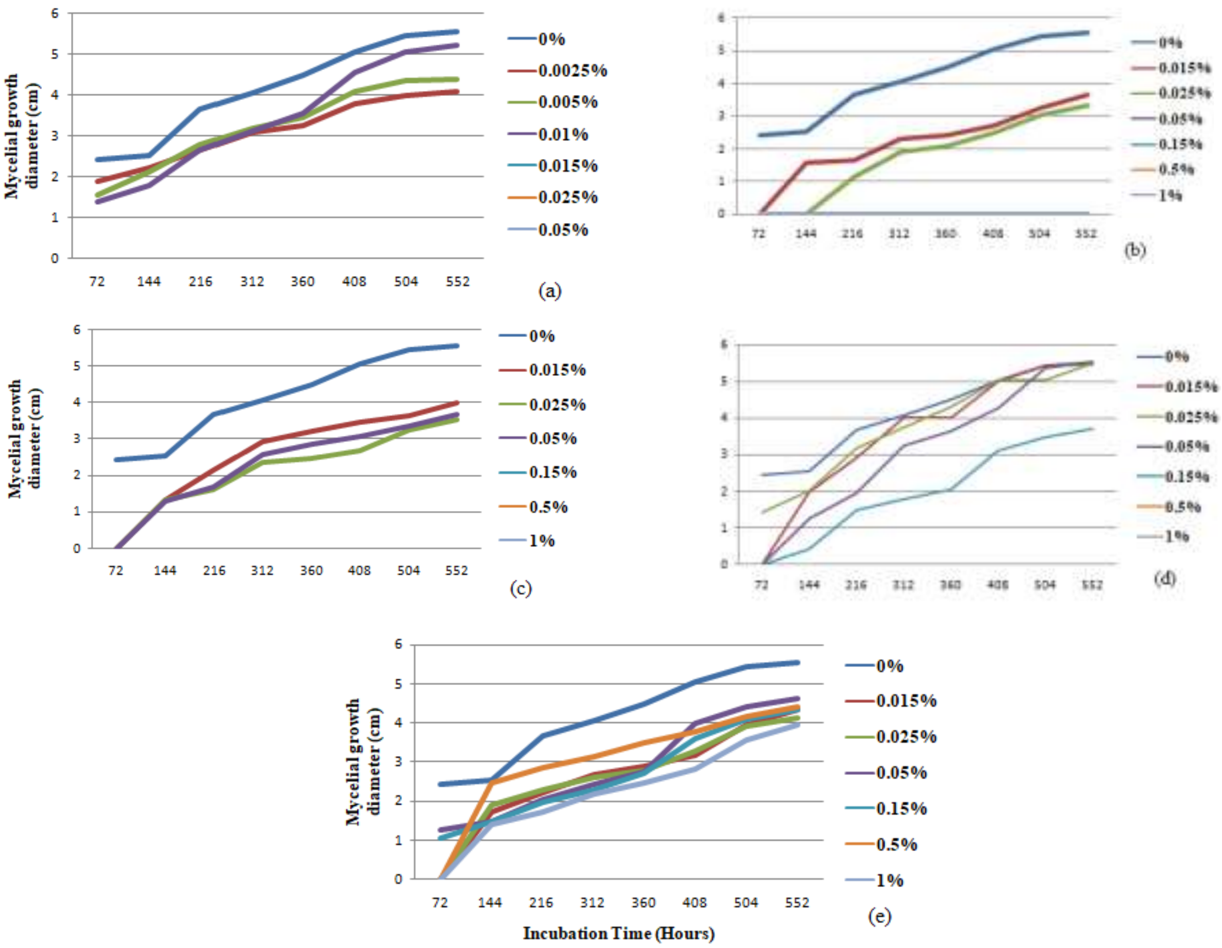

Figure 1. Effect of essential oils (a) Oregano, (b) Thyme, (c) Mint, (d) Myrtle, and (e) Eucalyptus on the mycelial growth of *A. rabiei in vitro.*

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thyme, mint, and myrtle.

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-Mondívil *et al*., (2006) demonstrated also an important antifungal activity of the essential oil of *T. vulgaris* against *Alternariacitri*. 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

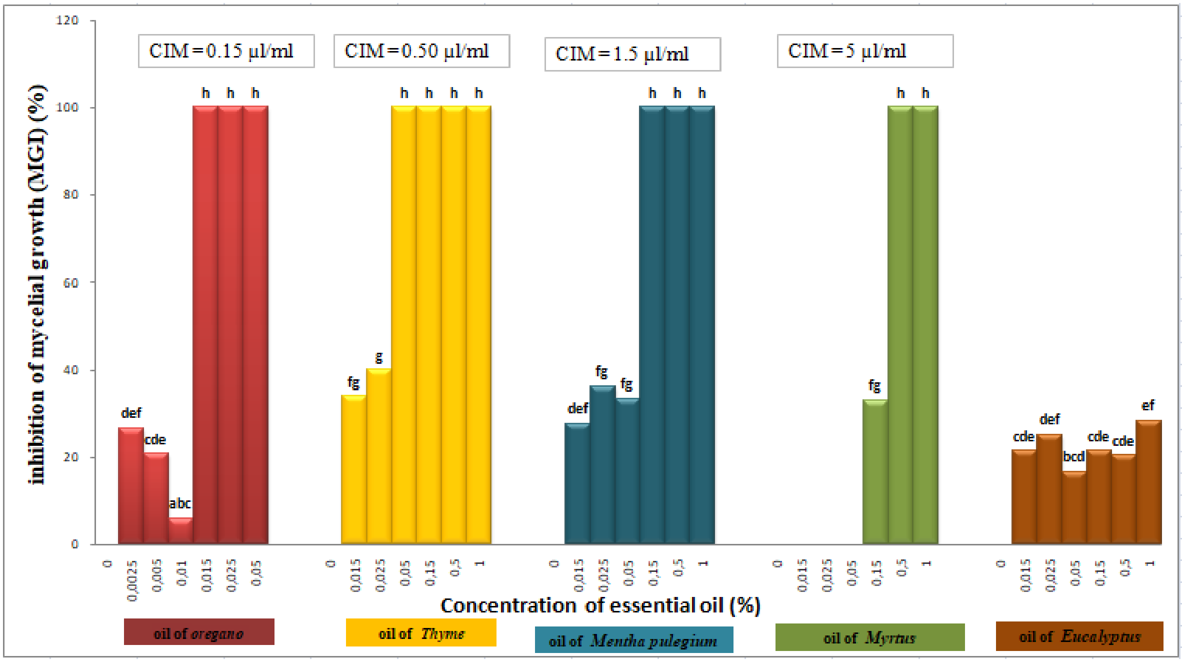

Figure 2. Effects of different concentrations of essential oils of oregano, thyme, mint, and myrtle on mycelial growth of *A. rabiei*. Concentrations with the same letters represent values that are not significantly different (P≤ 0.05).

Table 2. Property of antifungal activities of essential oils on growth inhibition of *A. rabiei*in new media of CPMA.

Essential oils	Concentration (µL mL^{-1})	Mycelial growth diameter (cm) in the new media
O. compactum	0.15	0
	0.25	0
	0.5	0
T. vulgaris	0.5	0
	1.5	0
	5	0
	10	0
M. pulegium	1.5	0
	5	0
	10	0
M. communis	5	2.5
	10	2

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 ($P\leq 0.05$) 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, 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

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Table 3. Phytotoxic effect of essential oils on germination of chickpea seeds. [a]

Essential oils	Concentration ($\mu L\ L^{-1}$)	G (%)	GI	MGT	SVI
Control	0	100^{d}	4.74^{cd}	5.78^{d}	618.1^{d}
Oregano	15 (MIC)	99^{d}	$5,01^{cd}$	4.81^{c}	561.8^{d}
	30 (MIC×2)	88^{c}	4.41^{c}	4.39^{c}	178.3^{b}
	150 (MIC×5)	66^{b}	3.53^{b}	3.25^{b}	61.5^{a}
Thyme	50 (MIC)	96^{cd}	5.15^{d}	4.52^{c}	304.7^{c}
	100 (MIC×2)	71^{b}	3.86^{b}	3.37^{b}	77.8^{a}
	500 (MIC×5)	0^{a}	0^{a}	0^{a}	0^{a}
Mint	150 (MIC)	0^{a}	0^{a}	0^{a}	0^{a}
	300 (MIC×2)	0^{a}	0^{a}	0^{a}	0^{a}
	1500 (MIC×5)	0^{a}	0^{a}	0^{a}	0^{a}
Myrtle	500 (MIC)	0^{a}	0^{a}	0^{a}	0^{a}
	1000 (MIC×2)	0^{a}	0^{a}	0^{a}	0^{a}
	5000 (MIC×5)	0^{a}	0^{a}	0^{a}	0^{a}

[a] 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).

Compound [a]	RT [b]	RI [c]	Oregano	Thyme	Mint	Myrtle	*Eucalyptus*
					% peak area		
Anisole	3.10	923	-	-	-	-	1.31
α-thujene	3.28	925	-	1.76	-	-	-
Tricyclene	3.41	926	-	-	-	-	1.14
α-Pinene	3.58	931	-	-	-	13.22	-
β-Pinene	6.58	974	-	1.63	-	-	-
α-Terpinene	8.50	1015	-	3.25	-	-	-
p-Cymen	9.96	1023	5.82	15.59	-	-	10.56
Limonene	10.13	1027	-	-	-	-	4.51
1,8-Cineole	11.10	1036	-	-	-	48.81	34.22
γ-Terpinène	11.34	1057	17.56	22.25	-	-	-
Linalol	12.94	1100	-	1,79	-	-	-
4-Terpineol	15.85	1176	2.15	1,15	-	-	3.84
Myrtenal	16.79	1197	-	-	-	-	11.34
Nopol	16.30	1212	-	-	-	-	2.66
Careen (2)	17.50	1227	-	-	-	-	1.01
Carvone	18.82	1231	-	-	-	-	2.81
Thymolmethylether	19.03	1233	-	1.18	-	-	-
Carvacryl methyl oxide	19.96	1244	5.05	-	-	-	-
Geraniol	20.40	1245	-	-	14.72	-	-
Pulegone	20.42	1247	-	-	84.75	-	-
Thymol	21.50	1293	25.9	41.39	-	-	-
Carvacrol	21.87	1311	38.67	2.06	-	-	-
Myrtenyl acetate	23.20	1328	-	-	-	36.67	-
Caryophyllene	26.76	1417	-	1.3	-	-	-
Guaiol	42.54	1596	-	-	-	-	6.28
Cedrol	42.52	1607	-	-	-	-	16.13

[a] The compounds that present lower than 1% are not shown. [b] Retention Time (Min). [c] Retention Indices on the HP 5MS column.

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Khatamsaz, 2003; Naghdi-Badi *et al*., 2004; Nickavar *et al.*, 2005; Hudaib and Aburjai, 2007; Amiri, 2012; Bahreininejad *et al.*, 2013). 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.

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REFERENCES

1. Abdelgaleil, S. A. M., Zoghroban, A. A. M., El-Bakry, A. M. and Kassem, S. M. I. 2019. Insecticidal and Antifungal Activities of Crude Extracts and Pure Compounds from Rhizomes of *Curcuma longa* L. (Zingiberaceae). *J. Agr. Sci. Tech.*, **21(4):** 1049-1061
2. Abdul-baki, B.A.A., and J.D. Anderson. 1973. Relationship between Decarboxylation of Glutamic Acid and Vigor in Soybean Seed. *Crop Sci.* **13**:222–226.
3. Ait-Ouazzou, A., Lorán, S., Arakrak, A., Laglaoui, A., Rota, C., Herrera, A. and Conchello, P. 2012. Evaluation of the

Chemical Composition and Antimicrobial Activity of *Mentha pulegium*, *Juniperus phoenicea*, and *Cyperuslongus* Essential Oils from Morocco. *Food Res. Int.,* **45(1):** 313–319.
4. Akamatsu, H. O., Chilvers, M. I., Kaiser, W. J. and Peever, T. L. 2012. Karyotype Polymorphism and Chromosomal Rearrangement in Populations of the Phytopathogenic Fungus, *Ascochyta rabiei*. *Fungal Biol.,* **116(11):** 1119–1133.
5. Amini, K. and Bahramian, S. 2019. Antifungal Activity of *Pistacia eurycarpa Yalt*. Essential Oil on *Aspergillus flavus* by Direct Addition and Vapor Contact. *J. Agr. Sci. Tech.,* **21(2):** 323-330
6. Amiri, H. 2012. Essential Oils Composition and Antioxidant Properties of Three *Thymus* Species. *Evid. Based Complement Alternat. Med.,* **2012**: 1–8
7. AOSA. 1983. Seed Vigor Testing Hand Book. Contribution No. 32 to the Handbook on Seed Testing. Association of Official Seed Analysis, Springer field, IL, USA.
8. AOSA. 1990. Rules for Testing Seeds. *J. Seed Technol.,* **12**: 1-112.
9. Bahreininejad, B., Razmjoo, J. and Mirza, M. 2013. Influence of Water Stress on Morphophysiological and Phytochemical Traits in *Thymus daenensis*. *Int. J. Plant Product*, **7(1):** 155–166.
10. Bayan, Y., Yilar, M. and Onaran, A. 2017. Antifungal Effect of Bay Laurel (*Laurus nobilis*) and Myrtle (*Myrtus communis* L) Essential Oil on Chickpea Blight (*Ascochyta rabiei*). *Sch. Bull.,* **3(12):** 625-629.
11. Bayar, Y. 2018. Determination of Antifungal Activity of *Mentha spicata* L. Essential Oils against Different Isolates of Chickpea Blight Disease [*Ascochyta rabiei* (Pass) Labr.] Türkiye Tarımsal Araştırmalar Dergisi, **5(2):** 92-96
12. Baite, M.S., Dubey, S.C. 2018. Pathogenic variability of Ascochyta rabiei causing blight of chickpea in India. *Physiol Mol Plant P*, **102**: 122–127.
13. Bouhdid, S., Senhaji, N., Idaomar, M., Zhiri, A., Baudoux, D., Amensour, M. and Abrini, J. 2008. Antibacterial and Antioxidant Activities of *Origanum compactum* Essential Oil. *Afr. J. Biotechnol.,* **7:** 1563–1570
14. Boukhebti, H., Chaker, A.N., Belhadj, H., Sahli, F., Ramdhani, M., Laouer, H. and Harzallah, D. 2011. Chemical Composition and Antibacterial Activity of *Mentha pulegium* L. and *Mentha spicata* L. Essential Oils. *Der. Pharmacia Lett.,* **3:** 267–275.
15. Boulenouar, N., Marouf, A., Cheriti, A. and Belboukhari, N. 2014. Medicinal Plants Extracts as Source of Antifungal Agents against *Fusarium oxysporum f. sp. albedinis*. *J. Agr. Sci. Tech.,* **14:** 659-669.
16. Chavan, P. S. and Tupe, S. G. 2014. Antifungal Activity and Mechanism of Action of Carvacrol and Thymol against Vineyard and Wine Spoilage Yeasts. *J. Food Cont.,* **46:** 115-120.
17. Cherrat, L., Espina, L., Bakkali, M., Pagan, R. and Laglaoui, A. 2014. Chemical Composition, Antioxidant and Antimicrobial Properties of *Menth apulegium: Lavandula stoechas* and *Satureja calamintha Scheele* Essential Oils and an Evaluation of Their Bactericidal Effect in Combined Processes. *Innov. Food. Sci. Emerg.,* **22:** 221–229.
18. Combrinck, S., Regnier, T. and Kamatou, G. P. P. 2011. *In Vitro* Activity of Eighteen Essential Oils and Some Major Components against Common Postharvest Fungal Pathogens of Fruit. *Ind. Crop. Prod.,* **33**: 344–349.
19. Dellacassa, E., Menéndez, P. and Moyna, P. 1990. Chemical Composition of *Eucalyptus* Essential Oils Grown in Uruguay. *Flavour. Frag. J.,* **5:** 91–95.
20. Den Broucke, C. and Lemli, J. A. 1980. Antispasmodic Activity of *Origanum compactum*. *Planta Medica,* **38:** 317–331.
21. Dorman, H. G. and Deans, S. G. 2000. Antimicrobial Agents from Plants: Antibacterial Activity of Plant Volatile Oils. *J. Appl. Microbiol.,* **88(2):** 308-316.
22. Ellis, R. A. and Roberts, E. H. 1981. The Quantification of Ageing and Survival in Orthodox Seeds. *Seed Sci. Techno.,* **19:** 373-409.
23. Enzo, M., Laura, O., Elvira, L. and Luca, R. 2012. Activity of Some Essential Oils against Pathogenic Seed Borne Fungi on Legumes. *Asian J. Plant Pathol.,* **6:** 66-74.
24. Guenther, E. 1948. The Essential Oils. D. Van Nostrand Co. *Priceton,* **1:** 316-318.

25. Hammer K. A., Carson, C. F. and Riley, T. V. 1999. Antimicrobial Activity of Essential Oils and Other Plant Extracts. *J. Appl. Microbiol.,* **86**: 985-990.
26. Hudaib, M. and Aburjai, T. 2007. Volatile Components of *Thymus vulgaris* L. from Wild Growing and Cultivated Plants in Jordan. *Flavour. Fragr. J.,* **22:** 322–327.
27. Krimi Bencheqroun, S., SeidJ, A. and Kamel Lhaloui, S. 2014. Status of Food Legumes Diseases in Morocco. *11th Arab Congress of Plant Protection,* Amman, Jordan.
28. Lima, G., De Cartis, F., and De Cicco, V. 2008. Interaction of Microbial Biocontrol Agents and Fungicides in the Control of Postharvest Disease. *Stewart Postharvest Rev.,* **4:** 1-7.
29. Martínez-Romero, D., Guillén, F., Valverde, J. M., Bailén, G., Zapata, P., Serrano, M., Castillo, S. and Valero, D. 2007. Influence of Carvacrol on Survival of *Botrytis cinerea* Inoculated in Table Grapes. *Int. J. Food Microbiol.,* **115(2):** 144-148.
30. Naghdi-Badi, H., Yazdani, D., Mohammad Ali, S. and Nazari, F. 2004. Effects of Spacing and Harvesting Time on Herbage Yield and Quality/Quantity of Oil in Thyme, *Thymus vulgaris* L. *Ind. Crops Prod.,* **19:** 231–236
31. Nickavar, B., Mojab, F. and Dolat-Abadi, R. 2005. Analysis of the Essential Oils of Two *Thymus* Species from Iran. *Food Chem.,* **90**: 609–611.
32. Numpaque, A. M., Oviedo, A. L., Gil, J. H., García, C. M. and Durango, D. L. 2011. Thymol and Carvacrol: Biotransformation and Antifungal Activity against the Plant Pathogenic Fungi *Colletotrichum acutatum* and *Botryodiplodia theobromae. Trop. Plant Pathol.,* **36(1):** 003-013
33. Ouakouak, H., Chohra, M. and Denane, M. 2015. Chemical Composition, Antioxidant Activities of the Essential Oil of *Mentha pulegium* L, South East of Algeria. *Int. Lett. Nat. Sci.,* **39**: 49–55.
34. Padman, M. and Janardhana, G. R. 2012. Screening for Inhibitory Activities of Essential Oils on the Growth of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., the Causal Agent of Leaf Spot Disease of *Murraya koenigii* L. *Arch. Phytopathol. Plant Protect.,* **45:** 1575–1581.
35. Sajjadi, S. E. and Khatamsaz, M. 2003. Composition of the Essential Oil of *Thymus daenensis Celak. ssp. Lancifolius (Celak.) Jalas. J. Essent. Oil Res.,* **15:** 34–35.
36. Satrani, 2010. *Valorisation des Plantes Aromatiques et Médicinales du Maroc*. ISBN: 978-613-1-51855-3, Éditions Univ. Euro, 153 PP.
37. Saxena, N. P., Saxena, M. C., Johansen, C., Virmani, S. M. and Harris, H. 1996. *Adaptation of Chickpea in the West Asia and North Africa Region.* ICRISAT/ICARDA Publication, PP. 101-110.
38. Serrano, M., Martinez-Romero, D., Castillo, S., Guille´n, F. and Valero, D. 2005. The Use of Natural Antifungal Compounds Improves the Beneficial Effect of MAP in Sweet Cherry Storage. *Innov. Food Sci. Emerg. Technol*., **6:** 115-123.
39. Shanee, S., Tanveer, A., Javaid, M. M., Chaudhry, K. M., Aziz, A. and Khaliq. A. 2011. Phytotoxic Effects of *Euphorbia dracunculoides*: A Weed of Rainfed Chickpea-Chickpea Cropping System. *Spain J. Agric. Res.,* **9:** 580-588
40. Sharma, N. and Tripathi, A. 2008. Effects of *Citrus sinensis* (L.) Osbeck Epicarp Essential Oil on Growth and Morphogenesis of *Aspergillus Niger* (L.) Van Tieghem. *Microbiol. Res*., **163**: 337-344.
41. Soto-Mondívil, E., Moreno-Rodríguez, J. F., Estarrón-Espinosa, M., García-Fajardo, J. A. and Obledo-Vázquez, E. N. 2006. Chemical Composition and Fungicial Activity of the Essential Oil of *Thymus vulgaris* against *Alternariacitri. e-Gnosis,* **4(16):** 1-7.
42. Starović, M., Ristić, D., Pavlović, S., Ristić, M., Stevanović, M., Aljuhaimi, F., Svetlana, N. and Özcan, M. M. 2016. Antifungal Activities of Different Essential Oils against Anise Seeds Mycopopulations. *J. Food Safe. Food Qual.,* **67:** 72-78.
43. Thompson, D. P. 1989. Fungitoxic Activity of Essential Oil Components on Food Storage Fungi. *Mycologia,* **81**: 151-153.

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44. Umran H. K. and Bapeer. 2011. Allelopathic Potential of Myrtle, *Myrtus communis* L. upon Some Crops. *Baghdad Sci. J.*, **9(1):** 104-112.
45. Yilar, M. and Bayar, Y. 2019. Antifungal Potential Of Essential Oils *Of Salvia Officinalis* And *Salvia Tomentosa* Plants On Six Different Isolates Of *Ascochyta Rabiei* (Pass.) Labr. *Fresenius Environ. Bull.*, **28(3):** 2170-2175.

ترکیب شیمیایی اسانس ها و فعالیت ضد قارچی آنها در کنترل *Ascochyta rabiei*

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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*) در شیشه آزمایشگاه مورد بررسی قرار گرفت. این بیمارگر بذر بُرد (seed-borne) عامل بلایت آسکوچیتا (Ascochyta) است که به عنوان مرضی با بیشترین زیان اقتصادی روی نخود تلقی می شود. در این آزمایش، Oregano، Mint، Thyme،و Myrtle در غلظت های کم (0.15-5 µL/mL) رشد شعاعی *A. rabiei* را به طور کامل متوقف کردند. مهم ترین تاثیر از مصرف Oregano (با غلظت بازدارنده کمینه MIC برابر 0.15 µL/mL) و به دنبال آن Thyme (MIC =0.5 µL/mL) به دست آمد. آزمون گیاه سوزی (phytotoxicity) این اسانس ها روی جوانه زنی نخود چنین نشان داد که اسانس Oregano و Thyme در غلظت MIC اثر گیاه سوزی ندارند در حالیکه Mint و Myrtle می توانند درصد جوانه زنی بذر نخود را کاهش دهند. ترکیب شیمیایی اسانس های آزمون شده با استفاده از کروماتوگرافی گازی- طیف سنجی جرمی (GC-MS analysis) به دست آمد. نتایج تجزیه آشکار ساخت که در موثر ترین اسانس ها بیشترین مقدار مواد مربوط بود به دو ماده (Thymol و Carvacrol) که می تواند نمایانگر ماده فعال اصلی در کنترل بیمارگرباشد. بنا بر این، اسانس های Oregano و Thyme یا مواد عمده آنها را می توان برای برگپاشی روی نخود بر علیه آلودگی به بلایت آسکوچیتا مورد بررسی قرار داد.

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