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Chemical composition and antifungal properties of Oregano essential oils against the Moroccan soil-born pathogen *Fusarium culmorum*

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Abstract

In our study, the application of Oregano was investigated *in vitro* against ten isolates of *Fusarium culmorum* the causative agent of wheat root rot in Morocco. The chemical composition of essential oils extracted by hydrodistillation from the aerial parts of *Origanum onites* and *Origanum compactum* was analyzed by GC. The major components of *O. onites* Eo were Carvacrol (29,7%) and *p*-cymene (22,48%) while Thymol (26,25%) was the predominant constituent in *O. compactum EO*. The results of the antifungal activity revealed that both oils have an inhibitory effect against all tested isolates. *O. onites* and *O.compactum* Eos inhibited the growth of all isolates tested by 100% at the concentration of 1 µL.mL^{-1} and 1.2 µL.mL^{-1} respectively. They have the potential to be used as antifungal agents for the control of wheat Rot root.

Keywords: Essential oils; *Origanum onites* ; *Origanum compactum* ; antifungal activity, *Fusarium culmorum*

Introduction

Wheat cultivation has long occupied a central place in the Moroccan agricultural system in terms of area and production. Like the majority of cultivated plants, wheat is sensitive to different forms of biotic and abiotic stress, particularly in the current context of climate change.

Root rot is among the most widespread and damaging diseases of cereal crops. This disease has been increasing in incidence and severity in recent years and it is expected that climate change may make these diseases even more damaging to the wheat industry (1). *Fusarium culmorum* (W.G. Smith) Sacc. is one of the most important causal agents of root rot of wheat which can cause significant yield losses (2,3,4,5). It was reported that it is the most prevalent root rot fungus in Morocco (6, 7, 8). The harmful effect of the species of

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Fusarium consists of deterioration of the quality and quantity of yield and production of mycotoxins that can pose a threat to plants, animals, and humans (9). Most wheat cultivars are susceptible to Fusarium root rot disease. A few cultivars had partial resistance (5) and the identification of novel sources of resistance that can be exploited by breeders has been reported recently (10). Effective disease control requires the integrated application of the different methods (11).

The most common root rot management approaches include cultural practices and application of fungicides. Synthetic pesticides play a major role today in crop protection programs, and the need for pesticides is growing with increasing production intensification. However, due to the widespread use of synthetic fungicides, different side effects have been noticed, such as the development of resistance among the target microorganisms, toxicity to humans and animals as well as environmental contamination (12).

Therefore, it is necessary to seek new plant protection alternatives that are economical and environmentally acceptable. Several researchers have focused on developing alternatives to synthetic fungicides for controlling root rot diseases varying from the application of essential oils, plant extracts, and chemical elicitors (13, 14, 15, 16). The research of pesticide-free control methods of *Fusarium culmorum* has been raised and many studies have been carried out (17, 18, 19).

Many plant extracts and plant essential oils have shown a wide array of biological activities, especially antimicrobial effects on different groups of pathogenic organisms including soil-borne fungi (20, 13, 21, 14, 22, 15) In addition to their innovative approach considered to be environmentally safe, biodegradable, effective and economically practical (23, 24,25).

Oregano is one of the highly valued culinary and medicinal plants that have been used for centuries. *Origanum* genus includes many species, most of which are endemic or have a local distribution around the Mediterranean region (26, 27). In Morocco, this genus grows in the Rif, Tangier, Northern southern Morocco, Haouz, and the High and Middle Atlas (28). Among *Origanum* species, *O. compactum* is the most widely distributed species throughout Morocco; it is a perennial plant species endemic to Morocco and southern Spain (29). As for *O. onites*, it is a narrowly distributed eastern Mediterranean species, occurring mainly in Turkey and Greece (30).

Origanum genus has medical importance for its antimicrobial, antifungal, antioxidant, antibacterial, antithrombin, antimutagenic, angiogenic, antiparasitic anti-cancer, and antihyperglycaemic properties (31, 32, 33).

The essential oil content and its composition are one of the most important quality criteria for Oregano for all purposes. There are several studies to reveal the potential of essential oils as antifungal agents (34, 35, 21, 14, 36, 15).

Origanum onites (also called Turkish Oregano) has interesting biological effects; in addition to its significant inhibitory activity on fungal growth (37), it is a growth inhibitor of certain bacteria such as *Pseudomonas cichorii* (38) and it traps free radicals and inhibits the oxidation of linoleic acid (39).

It is known that thymol and carvacrol are the main active components of oregano essential oil (40, 41). The mode of action of carvacrol (2-methyl-5-(1-methylethyl)phenol) has received considerable research attention due to its use in flavorings, as well as an antibacterial agent or antifungal in food preservation methods (42, 43, 44). Thymol (5-methyl-2-1-methylethyl)phenol) is an isomer of carvacrol, having the hydroxyl group at a different location on the phenolic ring. The hydrophobic nature of carvacrol and thymol enables them to react with lipids in the cell membrane and mitochondria, making them permeable and causing leakage of the cell components (40).

Considering all these points, the present study was conducted to evaluate the *in vitro* fungicidal activity

of the essential oils of *Origanum onites* and *Origanum campactum* for controlling wheat root rot disease caused by *F. culmorum.*

Material and Methods:

Plant material and extraction of the EOs

Aerial parts from *Origanum onites* (*O.o*) and *Origanum campactum* (*O.c*) were dried in the shade and then submitted to hydrodistillation using a Clevenger-type apparatus (45). 100 g of each dried aerial plant was added to 2L distilled water into a 5L flask. The whole was heated until boiling for 5 h. The essential oils, after their extraction, were collected and dried with anhydrous sodium sulfate (Na2SO4), then recovered and stored at 4 °C in the dark until being analyzed.

Essential oil yields were estimated for each sample and expressed by (%); according to the formula of Marion et al., 1994 (46):

EO yield (%) = (weight of EO obtained by distillation/weight of dry biomass) x 100

Gas chromatography-mass spectrometry (GC/MS) analysis:

The chromatographic analysis of the essential oil was carried out using gas chromatography type Perkin Elmer Clarus® 580 coupled to a mass spectrometer type Perkin Elmer Clarus® SQ 8 S. The fragmentation was performed using electronic impact under the ionization energy of 70 eV, with a column Rxi®-5ms (phase of low polarity, Crossbond®-bond 5% diphenyl/ 95% dimethyl polysiloxane) of 30 m in length, internal diameter equal to 0.25 mm and the thickness of the film was 0.25 µm. The column temperature is programmed from 50 to 280°C at 8°C/min. The carrier gas is helium with a flow of 1 mL/min. Injection mode was fractional (leakage rate: 1/20 flow rate 50 ml/min). The identification of the different constituents was carried out through the comparison of their mass spectra with those of the reference products contained in the Computerized libraries available: NIST/EPA/NIH Mass Spectral Library Search (version 2.0 g) 2011, Wiley Registry of mass spectral data as well as those of the basis of spectral data Adams (47).

Pathogen cultures:

In this study, ten isolates of *Fusarium culmorum* (I1, I2, I3, I4, I5, I6, I7, I8, I9 and I10) were used. They were isolated from infected wheat roots collected from different areas of Morocco and cultured on a PDA medium at 25°C.

Antifungal activity:

Antifungal activity was determined by the agar plate method described by Remmal (48) and Satrani (49). The EOs of *O.o* and *O.c* were dissolved in 0.5% of Tween 80 (Merck, Germany) and added at 40-45 °C into pre-sterilized PDA to give final concentrations ranging from 0.2 to 1.2 µl.ml^{-1}. Only a PDA was used as a control. A 5mm diameter mycelial disk of the actively growing mycelium of each isolate of *F. culmorum* was placed in the center of the petri dish. To prevent evaporation of EOs, plates were sealed with Parafilm, and then incubated in the dark at 25 ± 2 C. Mycelial growth was measured daily until control plates were completely colonized with mycelium. Mycelial growth inhibition was calculated according to a formula by Pandey (50). Replications were considered simultaneously for each concentration of samples. All tests were repeated in triplicate.

Statistical analysis:

Statistical analysis was carried out using a completely randomized design and the data obtained were analyzed with one-way ANOVA and Duncan's test at 95% reliability using GenStat software (GenStat Release 22 (PC/Windows 10)).

The presented results are reported as means (±standard deviation) of three replicates.

Results

Yield and Composition of the essential oils of *O. onites* and *O. campactum*:

The hydrodistillation of the plants of *O.o* and *O.c* yielded liquid oils with a strong herbaceous odor characteristic of Oregano. The oil yield of *O.o* was 1.80% while that of *O.c* was 1.2%. The major components of the EOs tested were identified and assessed by GC technique. Table 1 lists the components, identified in both EOs.

According to the GS analysis results, 26 components were identified in each oil, representing about 93.8% for *O.o* and 92.8% for *O.c.* The 6 major components of *O.o* EO were Carvacrol (29,7%), *p*-Cymene (22,48%), ç-Terpinene (9,81%), Thymol (9,44%), α-Terpinolene (2,9%) and Linalool (2,32%) while *O.c* EO components were *p*-Cymene (32,28%), Thymol (26,25%), ç-Terpinene (5,44%), Benzene 1-methoxy-4-methyl-2-(1-methyl ethyl)- (5,11%), Carvacrol (4,68%) and Caryophyllene oxide (2,89%), respectively.

Effect of essential oils on mycelial growth of *F. culmorum*:

Both tested essential oils affected the growth of the ten fungal isolates (Table 2). The results of the antifungal tests revealed that the oils have an inhibitory effect against all of the tested isolates. This effect varies considerably depending on the isolate and the concentration of the oil used. There were significant differences between treatment and concentration of Eos.

The Eos of *O.o.* and *O.c*. inhibited the growth of all isolates tested by 100% at the concentration of 1 $\mu L.mL^{-1}$ and 1.2 $\mu L.mL^{-1}$ respectively. The results showed that the MIC of the EO of *O.o* was 0.4 $\mu L.mL^{-1}$ for the four isolates I2, I4, I6, and I9 and it was of the order of 0.6 $\mu L.mL^{-1}$ for I5, 0.8 $\mu L.mL^{-1}$ for I1 and 1 $\mu L.mL^{-1}$ for the isolates I3, I8 and I10. While the MIC of the EO of *O.c* was 0.8 $\mu L.mL^{-1}$ for the isolates I2, I3, I5 and I7; 1 $\mu L.mL^{-1}$ for I6, I9 and I10 and 1.2 $\mu L.mL^{-1}$ for the isolates I1, I4 and I8.

The data indicated that the isolates I2, I6, and I9 are the most sensitive to *O.o* EO since the lowest concentration inhibits mycelial growth by 70% and they were inhibited completely at a concentration of 0.4 $\mu L.mL^{-1}$. The isolates I1, I4, and I8 showed resistance to *O.c* EO, they needed the biggest concentration of 1.2 $\mu L.mL^{-1}$ to be inhibited completely.

According to the results, I3 was the most resistant to *O.o* EO, compared to the other isolates. It showed no inhibition until a concentration of 0.8 $\mu L.mL^{-1}$ while the same concentration of *O.c*. EO inhibited its mycelial growth. I5 was the most resistant to the lowest concentrations of both oils; it was not inhibited at all at 0.6 $\mu L.mL^{-1}$ of *O.o* but inhibited totally at 1 $\mu L.mL^{-1}$.

Table 1: Chemical composition of *Origanum onites* and *Origanum campactum* essential oils

RT	Components	Composition (%)	
		O. onites	*O. campactum*
6.629	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	0,94	0,78
6.788	Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl	1,23	1,25
7.100	Camphene	0,23	0,18
7.655	1-Octen-3-ol	0,91	1,43
7.801	3-Octanon	0,71	1,27
7.913	á-Myrcene	0,00	1,14
7.922	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	1,61	0,00
7.980	3-Octanol	0,00	0,17
8.472	**Cyclohexene, 1-methyl-4-(1-methylethylidene)-**	**2,90**	1,08
8.697	***p*-Cymene**	**22,48**	**32,28**
8.772	D-Limonene	0,57	0,72
9.339	**ç-Terpinene**	**9,81**	**5,44**
9.489	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1à,2á,5à)-	0,17	0,41
9.672	1-Nonen-3-ol	0,17	0,00
9.918	Benzene, 1-methyl-4-(1-methylethenyl)-	0,00	0,33
10.085	1,6-Octadien-3-ol, 3,7-dimethyl-	**2,32**	1,39
10.269	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-	0,57	0,00
11.044	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)	0,56	0,00
11.436	endo-Borneol	0,35	0,47
11.648	Terpinen-4-ol	0,86	1,31
11.748	Benzenemethanol, à,à,4-trimethyl-	0,22	0,45
11.886	à-Terpineol	2,25	1,75
12.820	**Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-**	2,30	**5,11**
13.516	**Thymol**	**9,44**	**26,25**
13.779	**Phenol, 2-methyl-5-(1-methyl ethyl)-**	**29,68**	**4,68**
15.963	Caryophyllene	1,71	0,78
16.172	p-tert-Butylcatechol	0,00	0,87
16.176	o-Methoxy-à,à-dimethylbenzylalcohol	0,35	0,00
18.519	**Caryophyllene oxide**	1,24	**2,89**
19.715	Alloaromadendreneoxide-(1)	0,00	0,26
23.079	Naphthalene, 1,2,3,4,4a,5,6,7-octahydro-4a-methyl-	0,17	0,00
25.527	Retinoicacid	0,00	0,17

RT: Retention time

Table 2: Mycelial growth inhibition (%) of the 10 isolates of *F. culmorum* by different concentrations of Oregano Essential oils

Isolates	EOs	Concentrations (µL.mL^{-1})					
		0,2	**0,4**	**0,6**	**0,8**	**1**	**1,2**
I1	**O.o**	41,56±0,68 f	53,15±1,23 gh	63,13±1,36 jklm	**100±0 uv**	**100±0 uv**	**100±0 uv**
	O.c	7,06±1,17 ab	18,43±1,36 c	30,59±1,17 de	54,23±2,24 ghi	72,55±3,4 nop	**100±0 uv**
I2	**O.o**	70,24±1,19 mno	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100±0 uv**
	O.c	60,71±1,19 ijkl	67,86±1,19 lmn	82,41±1,28 qrs	**100±0 uv**	**100±0 uv**	**100±0 uv**
I3	**O.o**	0±0 a	0±0 a	0±0 a	63,13±1,36 jklm	**100±0 uv**	**100±0 uv**
	O.c	2,23±0,2 a	6,25±0,65 ab	18,43±1,36 c	**100±0 uv**	**100±0 uv**	**100±0 uv**
I4	**O.o**	41,66±1,39 f	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100±0 uv**
	O.c	37,5±1,39 ef	51,39±1,39 g	76,39±1,39 opqr	86,57±2,12 st	93,05±1,39 tu	**100±0 uv**
I5	**O.o**	0±0 a	0±0 a	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100±0 uv**
	O.c	0±0 a	0±0 a	18,83±1,18 c	**100±0 uv**	**100±0 uv**	**100±0 uv**
I6	**O.o**	78,87±1,41 pqr	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100±0 uv**
	O.c	36,62±1,41 ef	69,01±1,41 mno	83,1±1,41 rs	86,74±0,74 st	**100±0 uv**	**100±0 uv**
I7	**O.o**	36,87±1,36 ef	60±2,04 hijk	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100 ±0 uv**
	O.c	7,06±1,18 ab	13,33±1,36 bc	43,93±2,43 f	**100±0 uv**	**100±0 uv**	**100±0 uv**
I8	**O.o**	26,64±0,27 d	40,67±0,35 f	54,96±0,31 ghi	69,89±0,18 mno	**100±0 uv**	**100±0 uv**
	O.c	0±0 a	6,28±0,02 ab	15,49±0,29 c	51,26±1,68 g	65,8±0,85 klmn	**100±0 uv**
I9	**O.o**	75,63±5,19 opq	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100±0 uv**	**100±0 uv**
	O.c	32,58±2,69 de	69,21±1,53 mno	57,43±42,22 ghij	88,72±1,27 st	**100±0 uv**	**100±0 uv**
I10	**O.o**	0±0 a	0±0 a	4,63±0,83 a	56,78±9,22 ghij	**100±0 uv**	**100±0 uv**
	O.c	2,15±0,24 a	5,59±1,23 a	17,83±1,89 c	41,23±2,19 f	**100±0 uv**	**100±0 uv**

The values are presented in the percentage of inhibition of mycelial growth and correspond to the average of three replications ± standard error. Values with different letters are significantly different ($p<0.05$) (AVOVA, Duncan test)

Discussion

The *in vitro* activity of the essential oils of *Origanum onites* and *Origanum campactum* is effective on the ten isolates of *Fusarium culmorum* isolated from infected wheat roots. Indeed, the growth of mycelial fungi was inhibited by both essential oils. The positive correlation observed between the inhibition rate and the different concentrations of each oil demonstrates the significant inhibitory activity against the fungus.

Our results are consistent with those reported by other studies on the antifungal activity of Oregano. Gormez (51) reported that the Essential oil of *Origanum onites* inhibited the growth of *Saprolegnia parasitica* at a concentration of $10\mu l.ml^{-1}$. The study of Chebli (52) showed that the essential oils of *Origanum compactum* and *Thymus glandulosus* inhibited the mycelial growth of *Botrytis cinerea*. As well as the study devoted to the antifungal activity of EOs of *Origanum* by Korukluoglu (53) showed that EO has significant inhibitory activity against the fungi *Alternaria aterna, Penicellium roqueforti, Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus, Fusarium semitectum, Fusarium oxysporum,* and *Mucorra cemosus*.

Several species from the Fusarium genus are economically relevant because, apart from their ability to infect and cause tissue destruction on important crops such as corn, wheat, and other small grains on the field, they produce mycotoxins on the crops in the field and in storage grains (54). That is why a lot of studies have been carried out to find a biological alternative to control the damage caused by these species. Essential oils revealed good potential as antifungal agents against *Fusarium spp.* (21, 14, 36, 15, 55).

The oil yield obtained in this study concerning both oils was different from that obtained in other works (56, 57). This difference can be interpreted by many factors. Indeed, several papers are available indicating that the essential oil content of the Origanum species and the concentration of its main components may strongly be affected by the harvest period (58, 59), the extraction processes, and especially the environmental conditions (60, 61)

As shown by GC analyses of the EOs, *O. onites* was mainly composed of Carvacrol and *p*-Cymene accompanied by other constituents at relatively low levels. Many studies have demonstrated that Carvacrol is the main component in the essential oil of Turkish Oregano (39, 59). However, the essential oil of *O. campactum* is mainly composed of *p*-Cymene and Thymol; which is substantially similar to that of the study conducted by Aboukhalid (57). The chemical components of the EOs of Origanum species are reported to vary qualitatively and quantitatively according to geographical location and environmental conditions (30, 62) with carvacrol and thymol being the most dominant components. This variety might lead to changes in pharmaceutical properties and biological activities (63).

The mechanism of the toxicity of phenols towards fungi is based on the inactivation of enzymes that contain the SH group in their active site (64, 65). Phenolic terpenes also work by binding to the amine and hydroxylamine groups of microbial membrane proteins, which cause the alteration of membrane permeability and the leakage of intracellular constituents (66, 67). The effect of essential oils was also reported by Fung (68) who thought it may be the result of phenolic compounds of essential oils altering microbial cell permeability by interacting with membrane proteins. This would cause the deformation of cell structure and functionality and permit the loss of macromolecules from their interior (69).

Phenolic components in the essential oil were the main source of antifungal activity. These results are supported by many studies. Adam (70) explained the antifungal activity of oregano Eos by its chemical composition rich in carvacrol and thymol. Indeed Sokovic (71) tested the effect of 4 oils (*O. onites, Satureja thymbra, Salvia fruticosa,* and *Salvia pomiferasubsp*) against 13 fungal species and they showed that the highest antifungal activity was

obtained by *O.o* and *S. thymbra* which are rich in Carvacrol. Karmen (72) also showed that among 22 chemical components derived from EOs, carvacrol, and thymol are the most active against the 2 fungi "*Coniophora versicolor* and *C.puteana*". Similarly, Oukhouia (73) found that among the 4 compounds tested, the highest anti-fusarium activity was obtained by Carvacrol and Thymol.

Conclusion

In the present study, it can be concluded that *O.o.* and *O.c.* essential oils showed an interesting antifungal activity *in vitro* against the phytopathogenic fungi *F. culmorum* and have the potential to be used as antifungal agents for the control of wheat Rot root. The antifungal activity of both essential oils can come from their phenolic components and/or from their interaction with other components. Moreover, each of an essential oil's components makes its contribution to the biological activity of the oil. However, further studies are required for the development of essential oils as potential antifungal agents.

Conflict of Interest: None

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