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CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF FOUR ESSENTIAL OILS AGAINST PHYTOPATHOGENS RESPONSIBLE FOR ROOT ROT OF WHEAT IN MOROCCO

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ABSTRACT

The essential oil (EO) isolated by hydro-distillation from the leaves, stems and flowers of Thymus satureoides, Origanum compactum, Mentha pulegium and Artemisia herba alba from Moroccan plants were analyzed by GC–MS. The major components were respectively borneol (26,45%) and thymol (12,79%) of EO from Thymus satureoides, carvacrol (43,97%) and p-Cymene (17,87%) of EO from Origanum compactum, pulegone (78,07%) of EO from Mentha pulegium and thujone (59,07%) and α-campholene aldehyde (12,71%) of EO from Artemisia herba alba L. Antifungal activity of these four oils were tested by poisoned food technique against two plant pathogenic fungi viz Bipolaris sorokiniana (Bs) and four Fusarium culmorum's isolates (Fc1, Fc2, Fc3 and Fc4). Minimum inhibitory concentration (MIC), minimum fungi static concentration (MFC) and median inhibitory concentration (IC50) for Isolates tested showed a highly significant variability in their reaction against the action of essential oils tested. Isolate Fc2 was the most resistant to the action of the four essential oils compared to other strains, with IC50 ranging between 0.147 and 4.496mg/mL, MIC from 0.29 to 9.11mg/mL and MFC between 0.57 and 9.11mg/mL. These results suggested that these essential oils could provide natural alternatives against Fc and Bs.

Keywords: Fusarium culmorum; Bipolaris sorokiniana; Wheat; Essential oils; antifungal activity.

1. Introduction

Crop losses due to fungal diseases can amount to 12% in developing countries (Bajpai *et al*., 2008). Fungal diseases caused by soil fungi induce a significant loss in yield and quality of crops (Kumar *et al*., 2014). In Morocco, Fc and Bs cause root rot, common root, spot blotch and scald in cereal crops (Murray and Brennan, 2009; Chekali *et al.*, 2011). Frequent using of wide variety of synthetic chemical compounds of high concentrations as antimicrobial agents against phytopathogenic fungi in agriculture (Bajpai *et al.*, 2008) and prevalence of resistant pathogenic strains (Chebli *et al.*, 2003).

The EO preparations that possess antimicrobial activities (Riccioni and Orzali, 2011) have been the subject of many investigations (Matasyoh et al., 2007). Again, EOs of some plants have recently been proven to be successful bio-control agent (Chutia et al., 2006 ; Sokovic and Griensven, 2006). It was observed these EOs from Origanum compactum against foodborne bacteria (Sbayou et al., 2014a), Mentha pulegium and Artemisia herba alba strongly inhibited bacterial growth (Sbayou et al., 2014b). But there is no report of the antifungal activity of these Eos in phytopathogenic fungi. Therefore, the present study was made to determine antifungal activity of these EOs against some phytopathogenic fungal species viz Fc and Bs, with emphasis on the possible future application of these EOs as alternative antifungal agents.

2. Material and Methods

2.1. Fungal strains

Five fungal isolates responsible for root rot of durum wheat crops were isolated from plant pathology (Agronomic Research Moroccan Institutes). Four isolates of *Fc* (Fc1, Fc2, Fc3 and Fc4) and one isolate of Bs were tested.

2.2. Essential oils and chemical composition

EOs were extracted by steam distillation from the leaves, stems and flowers from Moroccan plant Thymus satureoides, Origanum compactum, Mentha pulegium and Artemisia herba alba (SANTIS-sarl Company in Morocco). Analysis of the chemical composition was performed by gas chromatography (GC Ultra Trace) coupled to a mass spectrometer (MS Polaris Q ion trap). GC is equipped with a VB-5 column (5% phenyl methylpolysiloxane), 30m*0.25mm*0.25µm for detecting an electron ionization system with 70eV was used. The carrier gas was helium with a flow rate of 1.4 mL/min. Two molecules of the triazoles family (Tetraconazole and Hexaconazole), widely used in the fungal disease control (Chekali *et al.,* 2011), are used as positives controls.

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2.3. Determination of IC_{50}, MFC and MIC

The evaluation of inhibition percentage of EOs tested was performed using the technique reported by Soylu *and Soylu* (2010). IC_{50} was determined from the equations of dose-response curves of concentrations of essential oils tested (Kumar *et al.*, 2014). Minimum fungicidal concentrations (MFC) were determined by transferring the mycelium disc, which have not grown, on the PDA medium without essential oil (Soylu *and Soylu*, 2010). IC_{50} and MFC values obtained were the median of three replicates. The results of the average of three replicates were compared by analysis of variance (ANOVA) followed by comparison of means by the Duncan test at a probability of 5% by SPSS 22 software.

3. Results

Chromatographic profiles and mass spectra show that the four EOs have different chemical compositions; the major compounds vary according to each essential oil (Table 1). Carvacrol represents 43.97% of the total composition of the essential oil of *Origanum compactum*, followed by p-cymene (17.87%), which is a precursor of thymol and carvacrol, thymol (11.56%) and γ -terpinene (8.88%). Borneol (26.45%) and thymol (12.79%) were the major compounds of the *Thymus satureoides* essential oil. Pulegone composes 78.07% of essential oil of *Mentha pulegium*. Thujone is the major compound (59.07%) from essential oil chemical composition of *Artemisia herba alba* analyzed in this study, followed by α-campholene aldehyde.

Table 1: Chemical composition of essential oils tested

Compounds	Percentage of compounds			
	Origanum compactum	*Thymus satureoides*	*Mentha pulegium*	*Artemisia herba alba*
Carvacrol	**43.97**	1.51	-	-
p-Cymene	17.87	2.18	-	-
Pulegone	-	-	**78.07**	-
Thymol	11.56	12.79	-	-
Thujone	-	-	-	**59.07**
γ-Terpinene	8.88	1.61	-	0.32
Dodecamethyl-1,11-dihydrohexasiloxane	8.71	8.47	13.43	5.26
α-Campholene	-	-	-	12.71

aldehyde				
Caryophyllene	1.85	9.17	1.89	-
Carveol (fr.1)	1.8	-	-	-
α-Terpinene	1.15	0.5	-	-
α-Thujone	-	-	-	11.73
α-Pinene	1.05	5.19	0.70	1.02
Borneol	0.23	**26.45**	-	-
α-Terpinenyl acetate	-	10.99	-	-
Camphene	-	7.16	-	-
Santolina triene	-	1.93	0.8	-
Endobornyl acetate	-	1.87	-	-
Alloocimene	-	1.56	-	-
Artemisia triene	-	-	-	3.75
β-Cadinene	-	1.41	-	-
γ-Cadinene	-	1.35	-	-
Copaene	-	0.37	-	-
Isopulegone	-	-	1.45	-
p-Menthone	-	-	1.1	-
à-Cedrol	-	-	0.51	-
Sabinene	-	-	-	3.05
Germacrene-D	-	-	-	0.44
Patchoulane	-	-	-	0.3
Nopol	-	-	-	0.72
Cis-Sabinene hydrate	-	-	-	0.93
α-Muurolene	-	-	-	0.16
Thujol	-	-	-	0.13
Total	97.07	97.18	97.95	99.59

Significant differences are observed between EOs tested and positives controls (Table 2). IC_{50} values show that the essential oil of *Origanum compactum* exerts the most important antifungal activity compared to other EOs tested and positives controls, between 0.018mg/mL and 0.147mg/mL. The significant antifungal activity of this EO is due to the presence of the phenolic compounds carvacrol and thymol. EOs of *Mentha pulegium* and *Thymus satureoides* have an average antifungal activity, compared to other essential oils tested. Antifungal activity from EO of *Mentha pulegium* is represented by IC_{50} ranging of 0.034mg/mL to 0.578mg/mL and between 0.154mg/mL and 0.582mg/mL for EO of *Thymus satureoides.* EO of *Mentha pulegium* was more active than *Thymus satureoides.* EO of *Artemisia herba alba* exerts, compared to other essential oils, has lowest action against isolates tested, with IC_{50} ranging from 0.725mg/mL to 4.496mg/mL.

Isolates tested showed a highly significant variability in their reaction against the action of EOs tested. Fc2 isolate resist to the action of the four essential oils tested with higher IC_{50}, MIC and MFC compared to other strains, IC_{50} between 0.147 and 4.496mg/mL, MIC ranging from 0.29 to 9.11mg/mL and MFC between 0.57 and 9.11mg/mL (Table 2). Fc4 isolate is less resistant than Fc2 to the antifungal action of the three essential oils, *Origanum compactum*, *Mentha pulegium* and *Thymus satureoides* while the most sensitive isolates are Fc1, Fc3 and Bs strain. Whereas the strain of Bs is less resistant than Fc2 isolate to the essential oil of *Artemisia herba alba*. Fc3 and Fc4 isolates are more sensitive to the action of this essential oil (Table 2).

Table 2 : IC_{50}, MIC and MFC (mg/mL) of EOs tested and positives controls

	Fungal isolates														
	Fc1			Fc3			Fc2			Fc4			Bs		
	IC_{50}	MIC	MFC	IC_{50}	MIC	MFC	IC_{50}	MIC	MFC	IC_{50}	MIC	MFC	IC_{50}	MIC	MFC
Essential oils															
Origanum compactum	0.018^{a}	0,07^{a}	0.29^{a}	0.044^{a}	0,14^{a}	0.14^{a}	0.147^{a}	0,29^{a}	0.57^{a}	0.074^{a}	0,14^{a}	0.29^{a}	0.046^{a}	0,14^{a}	0.29^{a}
Thymus satureoides	0.172^{c}	0,29^{b}	1.14^{b}	0.325^{c}	0,57^{b}	1.14^{b}	0.582^{b}	1,14^{b}	2.28^{c}	0.382^{b}	0,57^{b}	1.14^{b}	0.154^{b}	0,29^{a}	1.14^{b}
Mentha pulegium	0.071^{b}	0,57^{b}	1.14^{b}	0.169^{b}	1,14^{c}	1.14^{b}	0.578^{b}	2,28^{c}	4.56^{d}	0.327^{b}	1,14^{c}	1.14^{b}	0.034^{a}	1,14^{c}	1.14^{b}
Artemisia herba alba	1.9^{f}	2,28^{d}	4.56^{c}	0.725^{d}	1,14^{c}	1.14^{b}	4.496^{d}	9,11^{d}	>9.11	0.777^{c}	1,14^{c}	2.28^{c}	2.28^{d}	4,56^{d}	>9.11
Positive controls															
Triazoles															
- *Hexaconazole*	0.25^{d}	0.5^{b}	0.5^{a}	0.25^{c}	0.5^{b}	0.5^{a}	0.25^{a}	0.5^{a}	0.5^{a}	0.25^{b}	0.5^{b}	0.5^{a}	0.25^{b}	0.5^{b}	0.5^{a}
- *Tetraconazole*	0.625^{e}	1.25^{c}	1.25^{b}	0.625^{d}	1.25^{c}	1.25^{b}	0.625^{b}	1.25^{b}	1.25^{b}	0.625^{c}	1.25^{c}	1.25^{b}	0.625^{c}	1.25^{c}	1.25^{b}

NB. The values in the same column followed by the same letter are not significantly different according to Duncan test at 5% probability.

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Statistical analysis of IC_{50} shows no significant difference between the two isolates Fc2 and Fc4 towards essential oils of *Thymus satureoides* and *Mentha pulegium* while this difference is significant between the two other essential oils. IC_{50} values of *Origanum compactum* are no significant difference with *Mentha pulegium* EO for strain *Bs*, which exert the most important antifungal action on this strain compared to the other essential oils and positive controls used.

4. Discussion

Chemical composition of the EOs tested show that carvacrol, p-cymene, thymol and γ–terpinene were the major compounds of *Origanum compactum* EO, with some difference in the percentages of compounds (Chebli *et al.*, 2003; Oussalah *et al.*, 2006; Babili *et al.*, 2011) from several Moroccan regions. Borneol and thymol were the major compounds of the *Thymus satureoides* EO, and have been reported as major compounds of Moroccan EOs (Oussalah *et al.*, 2007;

Kloucek et al., 2012). Other chemotypes of Moroccan Thymus satureoides have carvacrol as major compound (El Bouzidi et al., 2013). Pulegone is a major compound of EO of Mentha pulegium as like plant from north of Morocco (Chraibi et al., 2016; Ait-Ouazzou et al., 2012) and contrary from Iran (Aghel et al., 2004; Mahboudi et al., 2008). Thujone and α-campholene aldehyde showed high percentages in the Artemisia herba alba EO. EO of Artemisia herba alba from Tunisian arid zone has also a chemical composition rich with Thujone (Mighri et al., 2010). Geographical origin of the raw material affects the chemical composition of EO (Oussalah et al., 2006; Al-Reza et al., 2010; Paolini et al., 2010). This composition can be affected by weather conditions, soil, species used and extraction technique (Szumny et al., 2010; Russo et al., 2013).

Antifungal activity showed a high significant difference between four EOs tested. The most important antifungal activity was obtained by *Origanum compactum* EO rich in phenolic compounds like carvacrol and thymol. These compounds were more active on several *Fusarium* strains (Dambolena *et al.*, 2012; Marei *et al.*, 2012). The synergy between the carvacrol and thymol leads to an improvement of EO action (Campos-Requena *et al*., 2015). *Thymus satureoides* and *Mentha pulegium* EOs exerts an average antifungal activity; that's corroborate with Zabka *et al.* (2014) and Hajlaoui *et al.*(2009). EO of *Artemisia herba alba* showed the lowest action, rich with thujone and poor in camphor and 1,8-cineol. Mighri *et al. (2010)* reported that camphor and 1,8-cineol are the most likely compounds to inhibit microbial growth.

Toxicity of the phenols containing in these EOs is primarily based on the inactivation of fungal enzymes containing the SH group in their active sites or by disturbing structure of fungal cell membrane (Hu *et al.*, 2017). On other hand, strains tested have shown a significant

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variability against action of tested essential oils, the most resistant isolate was Fc2 and Fc4. This resistance would be due to a selection of resistance strains on farmers' fields in which these strains were isolated. This selection would be induced by unreasonable use of chemical fungicides (Singh and Chhatpar, 2011), which is not the case on the two experimental stations where pesticides use respects the recommended doses, according to standards.

5. Conclusion

Four EOs tested *Thymus satureoides, Origanum compactum, Mentha pulegium* and *Artemisia herba alba*, showed variable antifungal activity towards three isolates of *Fc* and *Bs* strain. *In vitro* results suggest that these EOs can constitute a source of new fungicidal molecules, to be tested in the field to validate their effectiveness in the fight against wheat root rot.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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