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STUDY OF THE ANTIFUNGAL ACTIVITY OF *ORIGANUM COMPACTUM* ESSENTIAL OIL AGAINST DIFFERENT PLANT PATHOGENIC FUNGI

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Abstract

The resistance of fungal strains to synthetic products and the search for new alternatives to them are of great importance to consumers. The main objective of this study was to identify the chemical composition of the Essential Oil (EO) of Origanum compactum and to evaluate its anti-fungal properties against four species of fungi, *Fusarium culmorum, Fusarium solani, Alternaria alternata* and *Helminthosporium sativum*. The results of gas chromatography coupled with mass spectrometry (GC-MS) revealed the presence of three major compounds in the extract, Carvacrol (72.97%), ρ-Cymene (14.5%), and γ-Terpinene (6.01%). Antifungal activity was tested by the microdilution method on agar medium. EOs showed potent antifungal activity against all strains of fungi tested compared to controls with minimal inhibitory concentrations (MIC) ranging from 0.02% to 0.05%.

Key word: *Origanum compactum*, volatile extract, GC-MS, *Fusarium culmorum*, *Fusarium solani*, *Alternaria alternata*, and *Helminthosporium sativum.*

Introduction

Among Moroccan oregano species, two are widely exploited for marketing and industrial production: *O. compactum* and *O. elongatum* Morocco exports 97 tons of dried *O. compactum* Bakhy *et al.* (2014). Oregano species have recently aroused great interest, both in academia and in the food industry, as potential natural additives to replace synthetic products, in the United States and Europe Sbayou *et al.* (2014), Tepe *et al.* (2004), the phenolic compound carvacrol abundant in oregano oil has been approved as a food additive. Several research studies have shown that this phenolic compound is among the most effective plant antimicrobial agents known to date Sbayou *et al.* (2014), Ben Arfa *et al.* (2006), Baydara *et al.* (2004),

The *Origanum compactum* (Lamiacae family) is an endemic species of Morocco; it grows in the Rif, Tangiers, Northern Morocco, Central Morocco, Western Morocco, Southern Morocco, Haouz and Haut-Atlas. It is widely used in popular medicine because of its multiple therapeutic effects. It is recommended, among others, in the treatment of diarrhea, skin, urinary and respiratory infections, Belkamel *et al.* (2013). In Morocco, oregano is considered a panacea. It is mainly used as an infusion in the treatment of dysentery, colitis, gastrointestinal disorders, gastric acidity and bronchopulmonary disorders. Against colds, flu, and bronchitis, it is also administered as fumigation. It is also used against oral affections in addition it is a good appetite stimulant (Bellakhdar, 1997).

In the present study, we describe the chemical composition of the essential oil obtained from *Origanum compactum* cultivated in Morocco, and then we study the efficacy of the oil against phytopathogenic fungi.

Material and Method

Plant material and volatile extract analysis

The extraction of volatile compounds was obtained by steam distillation method from leaves of Origanum

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compactum collected in the North-West of Morocco on the outskirts of the Kenitra region, during the beginning of flowering period (the summer season 2018). Before the extraction the dried plant material is stored in the laboratory at room temperature. The volatile extract was dried under anhydrous sodium sulfate and stored in the dark at 4°C before analysis.

The studied Essential oil (Eos) was chemically analyzed by gas chromatography coupled to mass spectrometry (GC–MS) on a thermofischer capillary gas chromatograph coupled to the mass spectrometry system (GC ULTRA model S / N 210729). The analytical study is performed by injecting 1 µl of essential oil, using hexane as solvent (Derwich and Benziane, 2009), Ez-zriouli *et al.* (2019).

Fungal strains

The fungal species used in the experiments are *Fusarium culmorum* (F.C), *Fusarium solani* (F.S), *Alternaria alternata* (A.A), *Helminthosporium sativum* (H.S). The four agricultural pathogenic fungi were isolated from the roots of soft wheat affected by rot and harvested, and cultivated on potato dextrose agar (PDA) and were stored at 4°C.

Antifungal Test

The fungal strains were cultured on Agar dextrose medium enriched with variable proportions of the tested essential oils previously emulsified in a 0.2% agar-agar solution with sterile distilled water Remmal *et al.* (1993), Satrani *et al.* (2001). The mixture is poured at a rate of 15 ml in 90mm diameter Petri dishes. From a 7-days fungal culture on the PDA medium, a mycelial disc 6 mm in diameter is placed in the center of each Petri dish. After incubation of the plates at 25°C the diameters of the mycelium were measured after 24 hours of daily incubation until the 7th days. The reading is made in comparison with the control plates which are started under the same conditions and on the same day as the others.

The antifungal index which is determined by formula Sellam *et al.* (2014):

Antifungal Index (AI) = [1- (Da/Db)] x 100

- Da: growth diameter in treated tins (mm)

- Db: diameter of growth in control boxes (mm)

The Mycelial velocity for each concentration is determined by formula

Ez-zriouli *et al.* (2019):

VC = [D1/Te1]+[(D2-D1)/Te2]+ [(D3- D2)/Te3]+… + [(Dn-Dn-1)/Tin]

D = Diameter of the growth zone for each day (mm), Te = Incubation time (day).

Chemical composition of volatile extract

The volatile extract of Origanum compactum was extracted by steam distillation from dried leaves and analyzed by GC-MS. The oil yields were calculated on a dry weight basis as 3% (w/w). In fact this yield is higher than that found in Taounate 2.10±0.07% (Northern Morocco) Ramdan *et al.* (2015). And also to the different regions of Northern Morocco Mayesra (2.72%), Mharech (2.80%) and Bab Taza (2.86%) Laghmouchi *et al.* (2018). Studies carried out by the Forest Research Centre of Rabat on the volatile extract of O. compactum from the Rabat region have shown that the yield of essential oil and about 1.6% (Bellakhdar and Idrissi,1990) and to the Chefchaouen area (1.46 to 2.41%) Ghanmi *et al.* (2011). However, higher yields have been reported by other researchers in different regions of Morocco; Ben Karrich (4.10%) and Bni Ider (4.24%) Laghmouchi *et al.* (2018).

The identified combinations in volatile extract, retention time (RT) and quantitative percentage (%) of the compounds are summarized in (fig. 1 & table 1). A total of 7 compounds, amounting 99.99% of the volatile extract, were identified; the analysis showed that carvacrol (72.97%), was the main component in the volatile extract of Origanum compactum. Other major components were identified as ρ-Cymene(14.5%), and γ-Terpinene (6.01%).

Our results are in agreement with most of the authors who worked on the same species of oregano in northeastern Morocco precisely in the Beni Idder region, carvacrol (75.6%) is the main component followed by ρ-Cymene (8.3%) and γ-Terpinene (6.6%) Zantar *et al.* (2014). Other work also carried out in Morocco has proven the dominance of the same compounds in the essential oil of oregano Charai *et al.* (2011).

Similarly, studies carried out in Valencia (Eastern Spain) on samples of Mediterranean oregano have shown that the oil is dominated by carvacrol (43.26%) and thymol (21.64%), followed by ρ-cymene (13.95%) and γ-terpinene (11.28%) Pilar *et al.* (2015). In addition to the Taounate region (Northern Morocco) the essential oil of O.compactum collected in March 2013 is characterized by p-Thymol (47.85%), p-Thymol (15.74%) and γ-Terpinene (17.25%) as the majority compounds identified Ramdan *et al.* (2015).

Comparison of the chemical composition of the volatile extract of compact oregano in our sample with what is reported in the literature showed that the chemical

composition of EO is characterized by four main compounds: carvacrol, thymol, ρ-cymene and γ-terpinene. The qualitative and quantitative changes in the chemical are largely related to several factors, including topographical conditions that characterize the regions and areas where the plant material is collected, climatic (temperature, humidity) and edaphic conditions, plant age, genotype, maturity, harvesting period and procedure, conservation of the plant material, temperature and drying time, and extraction technique Laghmouchi *et al.* (2018), Ramdan *et al.* (2015) Greche *et al.*(2009), (Pibiri, 2005).

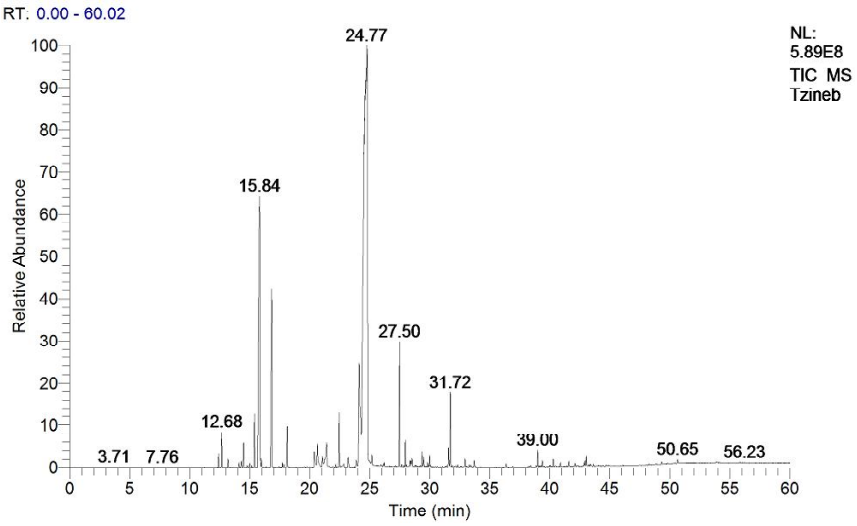
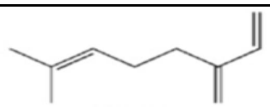
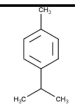
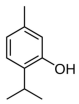
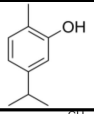
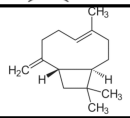
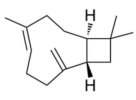


Fig. 1: GC-MS analysis of the *Origanum compactum* essential oil used in this work

Table1: Chemical composition of the essential oil of O.compactum

Chemical Compounds	Percentage (%)	Retention Time(RT) (min)	structural formula
ß-Myrcene	1.08	15.41	
ρ-Cymene	14.5	15.84	
γ-Terpinene	6.01	16.85	
Thymol	0.92	22.46	
Carvacrol	72.97	24.77	
Caryophyllene	2.61	27.50	
Caryophyllene Oxide	1.9	31.72	
Total	99.99		

In addition, degradation and transformation of some of the compounds may occur depending on the extraction the structure of chemical compounds may also be altered. The chemical composition depends on all these factors which can promote the biosynthesis of some molecules, and stop the synthesis of others (Bamola and Cedeno, 1999), (Boira and Blaquer, 1997), Rajeswara *et al.* (1993).

The results concerning the antifungal activity of essential oil, obtained by the agar dilution method, are presented in table 2. We found that the volatile extract of compact oregano showed very high inhibition of mycelium growth in all strains tested at very low concentrations. The minimum inhibition concentration (MIC) recorded showed that *Alternaria alternata*, *Fusarium culmorum* and *Helminthosporium sativum* are the fungi most sensitive than *Fusarium solani* to the presence of oil in the culture medium. Their growth was completely inhibited at a MIC of 0.02% while *Fusarium solani* resisted up to a MIC of 0.05%. The antifungal index values increased in parallel with the concentration of EO in the culture medium, generally exceeding 80% which clearly shows the excellent antifungal property of the oil against the four agricultural pathogenic fungal strains tested.

The growth kinetics of mycelium fig. 2 can be summarized as follows; the highest growth values are those recorded by the control: 2.34 mm/h, 1.097 mm/h, 1.09 mm/h, 0.45 mm/h for F.C, F.S, A.A and H.S respectively, then these values devoid at 0 mm/h in the presence of a low concentration of E.O (0.02%) in the culture medium for all

strains except F.S. where the rate reaches a value of 0.065mm/h at a concentration of 0.033% . This suggests that the presence of EO in the culture medium at different concentrations inhibits or blocks the growth of mycelium.

From these results it appears that the essential oil of *O. compactum* has a strong antifungal activity against all the agricultural pathogenic fungi tested. Because of the high content of constituents such as carvacrol (72.97 %), which is widely reported to have high levels of antifungal activity Ghanmi *et al.* (2015), Chebli *et al.* (2003), it has been suggested that it interacts with the cell membrane of the pathogen (Thompson, 1996).

And it's able to destabilize the cytoplasmic membrane and acts as a proton exchanger, thereby reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death Bouyaha *et al.* (2016), Ultee *et al.* (2002).

The correlation between the intensity of antifungal activity and the content of this constituent has already been reported by El Ajjouri *et al.* (2008). In other studies on the antifungal activity of oils from certain carvacrol-rich plants, namely *Thymus capitatus* (carvacrol 70.92%) against fungal strains, *T. versicolor, C. puteana, P. placenta* and *G. trabeum*. Results showed a high activity against these strains Panek *et al.* (2014), Karmen *et al.* (2003). Work has also shown the inhibitory effect of *Botrytis cinerea* in the presence of considerable amounts of carvacrol (Arras and Usai, 2001), Bhaskara *et al.* (1998). However, the compounds present in higher proportions are not necessarily responsible for the total activity; the involvement of less abundant constituents must also be taken into account Cimanga *et al.* (2002). In addition, antagonistic and synergistic effects may occur between oil components depending on the microorganism tested Cox *et al.* (2001).

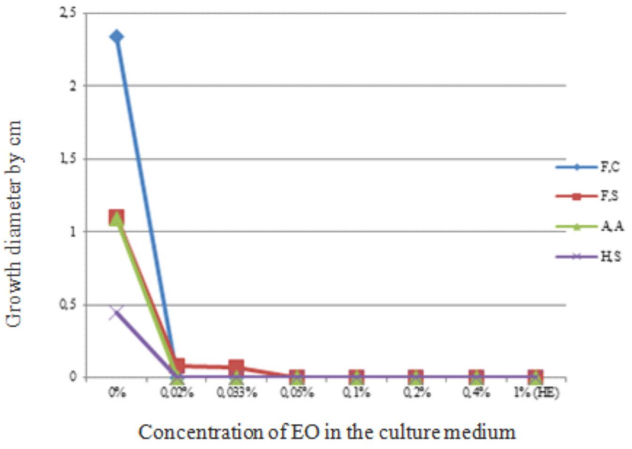


Fig. 2: Ratio of mycelia growth under the effect of different EO concentration of *Origanum compactum*

Conclusion

The exploitation of oregano has played and continues to play an important role in the daily lives of Moroccans. Its flavour is highly appreciated in various food preparations. Oregano is also useful for the preservation of many local food products, such as melted butter (S'men) and olives (Charai and Faid, 1999). It is frequently used in traditional medicine against digestive and pulmonary disorders (Bellakhdar, 1997), Remmal *et al.* (1993).

Several researchers have worked on extracts of compact oregano and their antimicrobial power but little research has focused on the secondary metabolites of essential oils and their powerful antifungal effect against

Table 2: Antifungal activity of *Origanum compactum EO*

Concentration en HE	oil growth(mm)				control growth(mm)				Antifungal index(%)			
	F.C	F.S	A.A	H.S	F.C	F.S	A.A	H.S	F.C	F.S	A.A	H.S
1%	0	0	0	0	84	79	77	39	100	100	100	100
0,4%	0	0	0	0	84	79	77	39	100	100	100	100
0,2%	0	0	0	0	84	79	77	39	100	100	100	100
0,1%	0	0	0	0	84	79	77	39	100	100	100	100
0,05%	0	0	0	0	84	79	77	39	100	100	100	100
0,033%	0	10	0	0	84	79	77	39	100	87,34	100	100
0,02%	0	11	0	0	84	79	77	39	100	86,08	100	100

F.C : Fusarium Culmorum; F.S : Fusarium Solani; A.A : Alternaria Alternata; H.S : Helminthosporium Sativum

agricultural fungal pathogens. As a result, *O. compactum* could be particularly advantageous in the fight against many species of fungi responsible for different phytopathogenic forms by using this essential oil in bio formulation which has many advantages over synthetic products. Other studies are necessary to estimate the toxicity of this oil *in vivo* in order to develop a natural means of biological control of fungal diseases that respect the environment.

Based on the above results, *O. compactum* essential oil could be proposed as an alternative fungicide. The development of such natural products would contribute to reduce the negative impact of synthetic agents as they can be effective, selective, biodegradable and more environmentally friendly. However, further studies will be needed to determine the effect of oregano EO on spore germination and *in vivo* tests will also be needed to assess their potential as preventive treatments. Similarly, it would be recommended to test the efficacy of carvacrol on resistant strains of plant pathogenic fungi.

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