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GC-MS analysis and *in vitro* study of antifungal, antiurease and anticholinesterase potentials of *origanum compactum* Benth. Essential oil

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Abstract

Origanum compactum is widely used as foods and in traditional medicine, considering the increasing use (culinary and medicinal) of this plant with beneficial effects on health and in search of new biomolecules with therapeutic effects. The current study aimed to investigate, the chemical composition of *O. compactum* essential oil prepared from the aerial parts and to provide data on its antifungal, anti-urease and anticholinesterase activities. *O. compactum* essential oil (EO) was analyzed by GC-MS, antifungal activity was carried out by the measurement of the radial growth of the fungus. Acetylcholinesterase (AChE), butyrylcholinestérase (BuChE) and urease inhibitory activity assays were used to determine enzyme inhibition capacity of the essential oil.

The GC-MS analysis revealed carvacrol (53.38 %) and thymol (21.16 %) as the major compounds. The essential oil exhibited an antifungal effect against *Fusarium oxysporum f.sp. lycopersici* (at 0.01% of the essential oil, 50.53 ± 2.13% of mycelia growth was inhibited). Urease activity was inhibited with an IC50 of 74.52±3.35 µg/ml. In addition, the investigation of anticholinesterase potential revealed a moderate acetylchlinesterase and butyrylcholinesterase inhibitory effects with an IC50 value of 103.25±1.86 µg/ml and 69.89±3.32µg/ml, respectively. This is the first study that demonstrates that essential oil can lead to inhibition of urease and butyrylcholinesterase enzymes.

According to these results, *O. compactum* essential oil could be a source of bio-fungicide, antiurease agents and an effective source of components having anticholinesterase activity.

Keywords: *O. compactum*, urease, antifungal, *Fusarium. Oxysporum*, AChE, BuChE.

1. Introduction

The Lamiaceae family is a family of plants widely distributed in the world; it includes more than 250 genera and nearly 7,000 species. Plants of this family have medical (traditional and modern), culinary, cosmetic and pharmaceutical uses (Harley, Atkins et al. 2004). The genus *Origanum* is a member of Lamiaceae family, including about 70 species, characterized by significant morphological and chemical diversity. Its most important area of distribution is the Mediterranean region. About 75% are found in the eastern Mediterranean and only a few species occur in the western part (Kokkini 1996; Skoula, Gotsiou et al. 1999; Baser 2002).

Oregano species have economic importance as a spice. They are also used in traditional medicine to treat health disorders. However, several studies have shown their antimicrobial (Gomez-Sanchez, Palou et al. 2011; Sözmen, Uysal et al. 2012), cytotoxic (Sivropoulou, Papanikolaou et al. 1996; Babili, Bouajila et al. 2011), insecticidal (Traboulsi, Taoubi et al. 2002), antioxidant (Lagouri, Blekas et al. 1993; Tepe, Daferera et al. 2004; Gortzi, Lalas et al. 2007; Nakiboglu, Urek et al. 2007; Ozkan, Sagdic et al. 2007; Babili, Bouajila et al. 2011), hypoglycemic (Lemhadri, Zeggwagh et al. 2004), antithrombin effects (Goun, Cunningham et al. 2002).

Origanum compactum Benth. is amongst the aromatic plants of the genus *Origanum*. Commonly known as zâatar, It is traditionally used by indigenous people in Algeria to fight against several illnesses such as digestive disorders, colds, coughs and whooping cough (Boulos 1983; Aissa 1999). In Moroco, *O. compactum*, as an endemic plant, is used to treat diarrhea, constipation, and diabetes (Bouhdid, Skali et al. 2008), gastrointestinal disorders, gastric acidity and bronchopulmonary disorders. It is also administered as fumigation (Bellakhdar 1997).

Regarding the bioactivity of this plant, several studies have reported antibacterial, antileishmanial, cytotoxic, antioxidant and antifungal properties. It has been shown that the *O. compactum* essential oil can be used as a food preservative (Ez-Zriouli, El Yacoubi et al. ; Babili, Bouajila et al. 2011).

The use of *O. compactum* by fumigation to treat colds prompted us to evaluate the anticholinestearse activity against acetylcholinesterase and butyrylcholinesterase, enzymes involved in alzheimer's disease (Ahmed, Khan et al. 2021). Moreover, its use in Morocco to treat gastrointestinal desorders, we assumed that the plant could have an inhibitory effect on the main enzyme secreted by *Helicobacter pylori* that is urease. it is important to point out that *H.pylori* responsible for the high gastric cancer rate (Vilaichone, Mahachai et al. 2013).

It has been demonstrated that the essential oil of *O.compactum* can be used as a food preservative (Ez-Zriouli, El Yacoubi et al.). Fusarium species are filamentous fungi pathogenic to plants of agronomic importance; they are mycotoxin producers and opportunistic human pathogens (Ilgen, Maier et al. 2008). The application of synthetic pesticides and fungicides is the main approach currently used to control fungi in agriculture. The intensive, uncontrolled and unregulated use of these chemical synthesis products can have harmful effects on the environment and public health (Kumari and John 2019). The search for a new natural source of biomolecule endowed with antifungal power was also the subject of our study. For this, the antifungal activity against *Fusarium oxysporum f. sp lycopersici* (FOL) was tested.

In brief, our study aims to find an important source of biomolecules with therapeutic effect on the one hand and on the other hand, to valorize a plant which grows in Algeria and of an economic and medical interest by determining the chemical composition of the essential oil and evaluating its biological activities: anticholinesterase, antifungal and antiurease.

2. Materials and Methods

2.1. Plant material and extraction of the essential oil

The aerial parts of *O. compactum* were harvested in June 2020 at flowering stage, in Hamam Ouled Ali region, 36°34'0" N et 7°24'0" E (Guelma, the North-East of Algeria, about 488 km of Algiers).

The *Origanum compactum* essential was procured from the company of Professor Lamouri Saad. The aerial parts of *O. compactum* were subjected to steam distillation. This method was chosen to increase the extraction yield. The essential oil obtained has been preserved in dark containers at 4°C for further experiments.

2.2. Chemical analysis of essential oil

2.2.1. Gas Chromatography (GC) analysis

The quantitative analysis was performed via a chromatographer in gas phase (**Hewlett Packard Agilent 6890 plus**) with HHP-5MS 5 % phenylmethyl, 95% dimethylpolysiloxane capillary column (30 m× 0.25 mm× 0.25 µm film thickness). The column temperature was programmed from 60°C for 8 min and then increased to 250°C at the rate of (2°C/min), isothermal for 10 min. The column temperatures of injector and detector were set at 250°C. Helium was used as the carrier, in a flow rate of 0.5 ml/min. The volume of injection was 0.2 µl. The percentage of each constituent in the oil was determined by area peaks.

2.2.2. Gas chromatography-Mass spectrometry (GC-MS)

The GC-MS analysis was carried out using gas phase chromatography (**Hewlett Packard Agilent 6890 plus**) coupled with mass spectrometer (Hewlett Packard Agilent 5973) equipped with ionization energy of 70 eV. The column temperature was programmed at: interface (280°C), source (230°C), and mass analyzer type was quadruple.

The compounds identification was achieved by comparison of their recorded mass spectra with Those of computer libraries (Wiley and Nist 11).

2.3. Biological activities

2.3.1. Anticholinesterase activity

The Cholinesterase inhibitory activity was qualitatively assessed using Ellman's methodology (Ellman, Courtney et al. 1961) with slight modifications. In this essay, two enzymes were tested, Acetylcholinesterase and Butyrylcholinesterase. Briefly, Acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates of the reaction. DTNB was used for the measurement of the cholinesterase activity. The reaction mixture contained 150 µl of 100 mM phosphate buffered saline solution (pH8.0), 10 µl of test sample diluted in ethanol in different concentrations and 20 µl of AChE (5.32_10-3 U) or BChE (6.85 _ 10-3 U) solution. The reaction

mixture was incubated for 15 min at 25 °C. Afterwards, 10 µl of DTNB (0.5 mM) and 10 µl of acetylthiocholine iodide (0.71 mM) or the butyrylthiocholine chloride (0.2 mM) were added to start the reaction. At 0 min and 15 min, the hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow5-thio-2-nitrobenzoate anion. This latest was the result of the reaction of DTNB with thiocholine released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, at a wavelength of 412 nm using the PherastarFS (BMG Labtech) detection system. Galantamine was used as a reference compound. All measurements were carried out in triplicate. The Percentage of inhibition I (%) was determined using the following formula (1):

$$\mathbf{I\ (\%) = (E - S) / E \times 100} \qquad \mathbf{(1)}$$

Where **E** is the activity of the enzyme without test sample (control), and **S** is the activity of the enzyme in the presence of the test sample. The IC50 values were calculated using the GNUPLOT package on line (www.ic50.tk, www.gnuplot.info).

2.3.2. In vitro Urease Inhibitory Analysis

Essential oil of *O. compactum* was evaluated for his urease inhibitory potential using (Taha, Ullah et al. 2018) method with some modifications. This method is based on the measurement of ammonia production by indophenol.

All reactions were performed in triplicate in a final volume of 200 µl and the reaction mixture comprising 25 µl of enzyme solution (5 U/ml), 10 µl of essential oil and the standard Thiourea at different concentrations (3.125 µg/ml – 200 µg/ml) and 50 µl of urea solution (17 mM). After an incubation of 15 min at 30 ˚C, 45 µl of phenol reagent (8% phenol and 0.1% w/v sodium nitroprusside), and 70 µl of alkaline reagent (2.85% NaOH and 4.7% active chloride NaOCl) were added at each concentration.

The experiment was carried out on 96-well microplate and the absorbance was read after 50 min at 630 nm using a multimode microplate reader (PerkinElmer, EnSpire, Singapore).

Percentage inhibition was calculated from the formula (2):

$$\mathbf{Urease\ inhibition\ \% = 100 - (A_{sample} / A_{control}) \times 100} \qquad \mathbf{(2)}$$

Where $A_{Control}$ and A_{Sample} are the absorbance values of the reaction medium in the absence and the presence of the inhibitor, respectively. The concentration of the sample inhibiting the enzyme by 50% (IC50) was calculated from the regression curves.

2.3.3. Antifungal activity study

The phytopathogenic fungi *Fusarium oxysporum f. sp lycopersici* (FOL) was tested for fungi toxicity by evaluating mycelial growth inhibition of phytopathogenic agent:

The measurement of the radial growth of the fungus on PDA medium (Potato, Dextrose, Agar), containing the oil to be tested was used as a method to determine the inhibitory activity of essential oil of *O. compactum*.

The experiment was carried out as follows; a volume of 1 ml of DMSO solution containing **1,25 µl, 2,5 µl, 5 µl, 10 µl and 20 µl** of the oil was added to 100 ml of PDA medium at 60 ° C, previously sterilized and then distributed in 4 petri dishes. Therefore, the final concentrations tested for each oil are: **0,00125%; 0,0025%; 0,005%; 0,01% and 0,02%**. Similarly, the positive control was prepared as the sample: 1 ml of DMSO was added to 100 ml of PDA medium. The negative control contains the PDA medium without any other products (Song, Zhou et al. 2004).

Experimentally, a disk of 5 mm in diameter is taken from a young fungal culture and is deposited aseptically in the center of the petri dish containing the PDA medium and the oil to be tested. The experiment is replicated 4 times (R1, R2, R3 and R4) for each concentration. After 6 days of incubation at 25 ° C, the mycelial growth of the phytopathogenic agent is measured at millimetric scale. Results were expressed as the percentage of growth inhibition of the fungus by each sample concentration with respect to the mean colony diameters of each fungus grown in control medium. Thus, the inhibition activity was expressed as a percentage and was calculated according to the formula (3) (Dennis and Webster 1971):

$$(\%)\ I=(C-T/C) \times 100 \quad (3)$$

Where I is the inhibition rate in%; C is the radial growth of phytopathogenic agent in mm on PDA medium with DMSO (control); T is the radial growth, in mm, of the phytopathogenic agent on PDA medium containing the oil to be tested.

Statistical analysis

The data were recorded as means ± standard deviation of three measurements. Analysis of variance was performed by ANOVA procedures. Significant difference was set at ($p < 0.05$).

3. Results

3.1. Chemical composition of the *O. compactum* essential oil

Thirty compounds were identified in the studied *O. compactum* EO by the GC-MS analysis accounting for 99.5% (listed in Table 1). The EO contained mainly oxygenated mono and sesquiterpenes, and mono and sesquiterpene hydrocarbon.

The main constituents *O.compactum* EO were Carvacrol (about 53.38%), Thymol (21.16%), p-Cymene (8.87%) and $_{\gamma}$-Terpinene (7.43%). α-Terpinene and α-Pinene are present with a percentage of 1.55 and 1%, respectively. All other compounds are present as minor components. The chemical structures of the major compounds of the EO are shown in Figure 1.

Table 1. Chemical composition of essential oil of *O. compactum*

N	Retention Time	Compound name	%
01	8.159	α-Thujene	0.44
02	8.577	α-Pinene	1.00
03	9.543	Camphene	0.07

04	11.223	β-Pinene	0.10
05	11.834	Octénol	0.04
06	12.029	B-Pinene	0.82
07	13.143	α-Phellandrene	0.26
08	13.858	α-Terpinene	**1.55**
09	14.629	p-Cymene	**8.87**
10	16.938	$_{\gamma}$-Terpinene	**7.43**
11	17.933	Bicyclo[2.2.1]heptane	0.20
12	18.659	A-Terpinene	0.08
13	20.168	d-linalool	0.86
14	21.025	Cyclohexanol	0.07
15	25.374	L-Borneol	0.10
16	25.900	4-Terpineol	0.36
17	29.901	Carvacrol methyl ether	0.19
18	34.731	Thymol	**21.16**
19	35.782	Carvacrol	**53.38**
20	38.674	p-Cymene	0.07
21	40.606	α-Gurjunene	0.68
22	41.452	Caryophyllene	0.19
23	42.635	Aromadendrene	0.40
24	42.635	Spathulenol	0.15
25	45.007	$_{\gamma}$-Muurolene	0.08
26	45.921	Viridiflorene	0.39
27	47.362	$_{\gamma}$-Cadinene	0.13
28	47.653	β-Cadinene	0.17
29	51.420	9,10-Dehydro-isolongifolene	0.16
30	69.286	Durenol	0.18
		Total	**99.58**

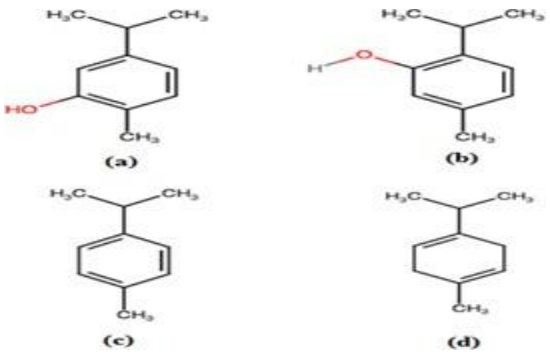

Figure 1. Chemical structure of main compound of of *O. Compactum* EO (1): Carvacrol, (2): Thymol, (3): p-Cymene (4): gamma-Terpinene

3.2. Cholinesterase Inhibition Assay of the EO

The anti-cholinesterase activity of the EO from *O. compactum* was evaluated on AChE and BuChE enzymes, using colorimetric method.

O. compactum essential oil acted as BuChE inhibitor with an IC50 of 69.89±3.32 µg/ml. On the other hand, the results also show that an inhibiting action was exerted on AChE enzyme with an IC50 of 103.25±1.86 µg/ml. Galantamine was used as a positive control and its IC50 value was 34.75±1.99 µg/ml (Table 2).

3.3. Urease Inhibitory activity

The results for the assessment of urease inhibitory activity of the EO are listed in Table 2. It was found that the *O. compactum* essential oil inhibited the urease activity at IC50 of 74.52±3.35 µg/ml. This result was compared to those of the positive control (Thiourea) which has an IC50 value of 11.57±0.68 µg/ml (Table 2).

Table 2. IC50 values of anticholinesterase activity and urease inhibitory activity of *O. compactum* essential oil and positive control (Galantamine and Thiourea).

IC_{50} µg/mL	Anti-cholinesterase		Anti-urease
	AChE	BuChE	
Essential oil	103.25±1.86[a]	69.89±3.32[a]	74.52±3.35[a]
Galantamine*	6.27 ±1.15[b]	34.75±1.99[b]	NT
Thiourea*	NT	NT	11.57±0.68[b]

Values expressed are means ± S.D. of three parallel measurements.

Values with different letters in the same column are significantly different ($p<0.05$)

*Reference compounds.

NT: not tested

3.4. Antifungal activity assay

Essential oil of *O. compactum* inhibited the mycelial growth of *F. oxysporum f.sp. lycopersici* (FOL) in dose dependent manner. A concentration of 0.02% of oregano essential oil almost completely inhibited the mycelial growth of fungus (% I was 94.6%). The results showed that approximately 50 percent of mycelial growth was inhibited at a concentration close to 0.01% (50.53±2.13 %) (Table 3 and figure 2).

Table 3. Percentage of growth inhibition of *O. Compactum* essential oil against *Fusarium oxysporum f. sp lycopersici* (FOL)

Oil concentration	0.00125%	0.0025%	0.005%	0.01%	0.02%
% of growth inhibition	3.2±00	5.09±2.17	9.25±1.36	**50.53±2.13**	94.65±1.35

Values expressed are means ± S.D. of three parallel measurements.

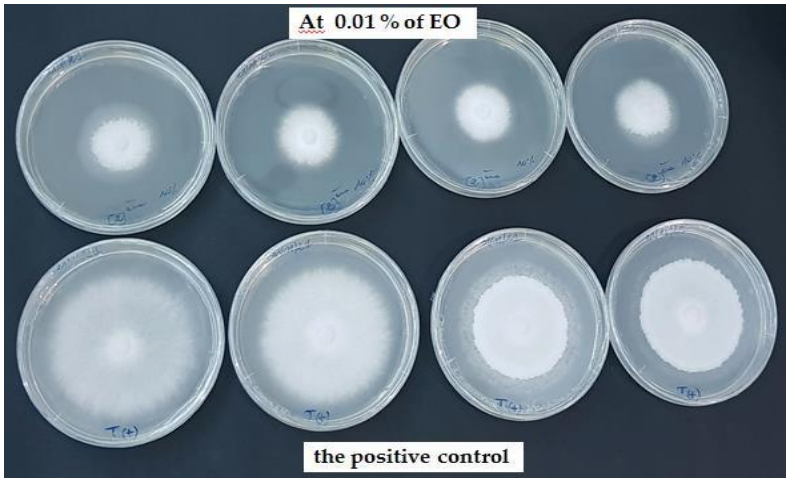

Figure 2. Percentage of mycelial growth of *Fusarium oxysporum f. sp lycopersici* at 0.01% of *O. Compactum* EO

4. Discussion

In the current investigation study, the chemical analysis, antifungal, anticholinesterase and urease inhibitory activities of *O.compactum* essential oil were studied.

The chemical analysis of *O. compactum* essential oil was studied by several studies conducted in Morocco. Our results are close to those found in the literature, the difference lies in the percentages of the compounds.

Many studies have shown that carvacrol and thymol are the main compounds of the essential oil tested followed by their biogenetic precursors γ-terpinene and p-cymene (Bakhy, Benlhabib et al. 2014; Chaouch and Chaqroune 2021; Aimad, Sanae et al. 2022). Our results are fully consistent with those reported in literature.

The literature has indicated that the chemical composition of *O. compactum* essential oil can be affected by environmental factors such as: rainfall, altitudes, soils, and the percentage of organic matter (Aboukhalid, Al Faiz et al. 2017). The harvesting period, the extraction method, and the drying of the plant can influence the yield and composition of *O. compactum* essential oil (Rezouki, Allali et al. 2021). Our results differ from those of the literature in percentage of the compounds; this difference is explained by the environmental factors and the experimental conditions.

Cholinesterase inhibitory activity of *O. compactum* was evaluated against two enzymes (AChE and BuChE).

L'acetylcholine (ACh) is a neurotransmitter that is found in the nervous system, it ensures the

activity and development of the cerebral cortex, control of blood circulation, control of sleep and wake cycles, and the process of learning and memory. Acetylcholinesterase (AChE) is the key enzyme hydrolysis of acetylcholine. Inhibition of acetylcholinesterase (AChE) prolongs the activity of acetylcholine in the transmission of nerve impulses.

In the treatment of diseases characterized by low levels of ACh, such as Alzheimer's disease, acetylcholinesterase and butyrylcholinesterase inhibition testing is important (Houghton, Ren et al. 2006).

With patients with Alzheimer's disease (AD), AChE activity remains unchanged or decreases, while BChE activity increases. Studies have shown that selective inhibition of BuChE increases the level of acetylcholine significantly, and also improves memory in aged rats (Ahmed, Khan et al. 2021). In the present study, we are looking for natural products of plant origin with a therapeutic potential to help in slowing down the evolution of this devastating illness. In this context, cholinesterase inhibitory activity of *O. compactum* was evaluated against two enzymes (AChE and BuChE).

The results showed that the essential oil tested was active against both enzymes (AChE and BuChE). For AChE, This is not the first time that this essential oil has been evaluated as an inhibitor of AChE (López, Cascella et al. 2018). Anti-butyrylcholinesterase activity has not been studied before. To the best of our knowledge, these results are new and here published for the first time.

It has been demonstrated that the carvacrol and thymol, have an effective inhibitory activity against both enzymes (AchE and BuChE) (Jukic, Politeo et al. 2007; Aazza, Lyoussi et al. 2011; López, Cascella et al. 2018).

Carvacrol and thymol were evaluated for their inhibitory activity of AChE in several studies, where they have shown their inhibition efficiency (Jukic, Politeo et al. 2007; Aazza, Lyoussi et al. 2011). It has been showed that carvacrol has a better ability to inhibit AchE enzyme than thymol and contrary to the anti-BuChE activity, it is the thymol which has a better activity (Kurt, Gazioglu et al. 2017).

Carvacrol and thymol are isomeric phenolic monoterpenes which can be found in several aromatic medicinal plants. The different position of the hydroxyl group in the phenyl ring provides a better anticholinesterase activity. These data lead us to say that the effectiveness of our essential oil against the two enzymes can be explained by the presence of the two major compounds, carvacrol and thymol.

Urease occurs in many plants, selected fungi, and a wide variety of prokaryotes. It is an enzyme which catalyzes the hydrolyzes of urea to produce ammonia and carbon dioxide on further decomposition (Amtul, Follmer et al. 2007).

Urease inhibitors can mitigate the negative role of urease in living organisms. They are effective against many serious infections caused by urease secretion by *Helicobacter pylori* which include gastric tract syndromes, urinary tract infections.

With the aim of looking for urease inhibitors, the essential oil *O. compactum* was screened for urease inhibition effect comparing with the standard compound, Thiourea. The tested essential oil showed a moderate inhibitory activity of urease with an IC50 of 74.52±3.35 µg/ml. These results are obtained for the first time with the essential oil of *O. compactum*, no study has been carried out before.

This inhibitory activity may be due to carvacrol, the main compound of the essential oil tested. This compound has been shown to have the ability to inhibit urease (Eftekhari, Ardekani et al. 2021).

Crop yield can be reduced by phytopathogenic fungi. Their structure of reproduction in spore allows their rapid propagation over vast cultivated areas. The spores are disseminated by wind, soil and water and can infect a large number of plants.

Unfavorable germination conditions can cause spores to lag for a long time. After infection and growth, fungi can produce enzymes and toxins that can damage plant structures and cause imbalances, and consequently, a decrease in yield or even a total loss of harvest (Fonseca, Lehner et al. 2015).

In agricultural practices, chemicals are frequently used to control plant pathogenic microorganisms. Many problems have been reported due to chemical products such as: environmental contamination, increased production costs and resistance of pathogens to the treatments. These are one of the current challenges we are facing (Joshi 2013).

To minimize these impacts, the use of natural products as an alternative is widely studied. The effectiveness of plant extracts and essential oils (EO) in the control of pathogens resistant or not to chemical products has been shown (Fonseca, Lehner et al. 2015).

In this context, our study was carried out to study the inhibiting effect of *O. compactum* essential oil on the mycelial growth of *Fusarium oxysporum f.sp. lycopersici* (FOL) fungus.

In this study, the studied fungus (*fusarium oxysporum f.sp. lycopersici* (FOL) was isolated and identified by Debbi, et al. (Debbi, Boureghda et al. 2018). It was isolated from tomato cultivation fields in Algeria.

The results showed that the essential oil of oregano (*O. compactum*) has an inhibitory effect on the mycelial growth of FOL. At a concentration of 0.01%, 50.5 % of the growth was inhibited.

The inhibiting effect of the essential oil of *O. compactum* on fungi has already been demonstrated in other studies. The antifungal activity carried out by (Aimad, Sanae et al. 2022) against Candida albicans, Aspergillus flavus, Aspergillus niger and *Fusarium oxysporum* revealed a wide antifungal spectrum of the studied essential oil against the tested strains. The MIC was 12.5 µg/ml) against *Fusarium oxysporum*.

(Santamarina, Roselló et al. 2015) study showed that the *O. compactum* essential oil has the best antifungal activity towards Fusarium species and Bipolaris oryzae with a total inhibition of the mycelial growth.

These studies and our study confirm the inhibitory effect of *O. compactum* essential oil on the phytopathogenic fungus *fusarium oxysporum f.sp. lycopersici*.

The chemical composition of EOs is closely related to their antifungal effect. Monoterpenes and sesquiterpenes with aromatic rings and phenol groups are able to form hydrogen bonds with the active sites of target enzymes (Belletti, Ndagijimana et al. 2004). EOs cause cell membranes damage, causing leakage of cellular materials and ultimately the microorganism death (Cox, Mann et al. 2000).

The antifungical effect of carvacrol and thymol, the main compound of Oregano essential oil has been found in several studies such as (Bouchra, Achouri et al. 2003) and (Zhao, Yang et al. 2021). It has been demonstrated that the carvacrol (main coumpound of Origano oil) is able to destabilize

the cytoplasmic membrane and acts as a proton exchanger, thereby reducing the pH gradient across the cytoplasmic membrane and lead to cell death (Ultee, Bennik et al. 2002), which explains the inhibitory activity of our essential oil.

5. Conclusions

The essential oil of the areal part of *O. compactum* collected in Guelma (North West of Algeria) was analyzed for the first time by GC-MS. The most abundant components were carvacrol (53.38%) and thymol (21.16%). Anticholinesterase, antifungal and anti-urease activities were also tested. Moreover, the EO of *O. compactum* exhibited an interesting, selective inhibitory activity against the AChE , BuChE and urease enzymes. The mycelial growth of FOL was also inhibited. The originality of this study is the evaluation of the inhibitory activity of the BuChE and urease enzymes and chemical analysis of essential oil of *O. compactum* from Algeria.

These results showed the variety of actions exerted by *O. compactum*, which make the plant a potential source of bioactive substances with various applications.

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