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Full Length Research Paper

A study on the determinant of chemical composition of the essential oils of Algerian citrus

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The aim of this study was to determine the chemical composition of the essential oils of Algerian citrus. They were extracted by hydrodistillation from the leaves of citrus species (orange, Bigaradier, mandarin and lemon), using gas chromatography/mass spectrometry (GC/MS). Their chemical composition and antifungal activity against four phytopathogenic fungi (*Fusarium oxysporum f.sp., albedinis sp, Penicelium* sp*., Alternaria* sp. and *Fusarium* sp.) were studied. The inhibiting minimal concentration (CMI) effect was also given for four oils. Ten compounds were recorded jointly among the 51 identified, of which limonene (7.18 to 36.10%), β-pinene (4.35 to 30.0%) and linalool (0.21 to 63.03%) represent the principal major compounds. These results indicate that essential oils can be employed as natural fungicides against phytopathogenic fungi.

Key words: GC/MS, essential oils , citrus, antifungal activity, phytopathogenic fungi.

INTRODUCTION

Fungal diseases are considered as the main enemies of crops. Apart from the fact that they have the potential to cause significant yield losses and deterioration of agricultural products, many of them cause a very serious risk to consumers because they produce dangerous toxins. Control methods applied involved mainly the application of chemical fungicides. In recent years, the polemic against the use of harmful chemicals on people' health and the environment has generated a debate on being exposed to the risk of having cancer and residual toxicity (Yaouba et al., 2010).

The increasing consciousness of consumers on the relationship between mode and health revolutionizes food, to justify the search for new strategies and to explore other surer solutions for replacement and biodegradable industry (Combrinck et al., 2010). For the elimination of pathogenic micro-organisms in the past years, researchers were interested in composing biologically active isolates, starting from plants with the objective of providing solutions to several challenges facing producers and agricultural distributors (Combrinck et al., 2010).

Essential oils are of a great interest because of their broad acceptance by consumers and the exploitation of their potential multipurpose utilities (Sawamura, 2000). Several researches evoked the antifungal activity of

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essential oils extracted from citrus (Mishra and Dubey, 1994; Sherma and Tripathi, 2006; Espina et al., 2011). The studies reveal that essential oils and their components have a very significant antimicrobial potential.

The studies were devoted to the determination of the chemical composition of the species of Algerian citrus (Baaliouamer, 1987). The antifungal study of the capacity of these essential oils constitutes a very promising alternative *vis-a-vis* the problems connected to the environment and the health of the consumers caused by the use of synthetic fungicides. Consequently, the objectives of this study were to determine (A) the chemical composition of essential oils of orange *(Citrus sinensis* (l) Osbeck), Bigaradier *(Citrus aurantium*), lemon *(Citrus limonum*) and mandarin *(Citrus reticulata*) by GC/SM, in order to identify the principal molecules responsible for inhibition, (b) the capacity of the antifungal activity of essential oils of the citrus against *Alternaria* sp*. (*A. sp*), Fusarium oxysporum f.* sp*. albedinis (*F. Ox*), Fusarium* sp*.* (F. sp) and *Penicelium* sp*.* (P.sp).

MATERIALS AND METHODS

Samples

Essential oils were extracted from the fresh leaves of orange, mandarin, Bigaradier and lemon from the Citrus Orchards of Chlef, Algeria in March - November 2011.

Standard cultures

Essential oils were individually examined in respect to four phytopathogenic fungi. The strain, *F. Ox was* provided by the Laboratory of the Pathology of Plants, the Institute of Agronomy, University of Chlef. The other three fungal strains, *A.* sp*., F.* sp. and *P.sp*, were from the Laboratory of Mycology of INPV Chlef. All the stocks were purified and maintained on potato dextrose agar (PDA) medium at 22 ± 2°C.

Essential oil extraction

Essential oils are obtained from the fresh leaves of orange, Bigaradier, lemon and mandarin by hydrodistillation for 3 h, using a Clevenger type apparatus. The Eos obtained are dried over anhydrous Na_2SO_4 and stored in sealed vials at 4°C until analysis

GC/MS analysis

GC/MS analysis was performed on a Hewlett Packard 6890 Series II capillary gas chromatography directly coupled to the mass spectrometer system (Agilant of the type 5973). HP-5MS non polar fused silica capillary column (50 m x 0.32 mm; 1.25 µm film thickness) was used under the following conditions: oven temperature program was from 60°C (2 min) to 260°C at 5°C/min and the final temperature was kept for 10 min; injector temperature, 250°C; carrier gas He, flow rate, 1 ml/min; the volume of injected sample was 0.1 µl in splitess mode during 0.1 min ionization energy 70 eV, in the electronic ionization (EI) mode; ion source temperature, 230°C; scan mass range of m/z 40 to 650 and interface line temperature, 280°C. The constituents of essential oils were identified based on their Kovats Index; they were calculated in relation to the retention time of a series of alkanes (C7 to C29) used as reference products, compared to those gathered by Adams (2001). The similarity of their mass spectra with those gathered in the NIST-MS library or reported in the literature was compared.

Antifungal activity

Analyzing mycelia growth

The inhibiting capacity of essential oils was examined. F. Ox, *P sp, A. sp* and *F. sp were* screwed using food poisoning technique (Grover and Moore, 1962). Necessary quantities of essential oils are separately dissolved in 0.5 ml of 5% (v/v). Tween-80 was added to various sterilized Petri dishes (9 X 1.5 cm) containing 9.5 ml of PDA medium in order to provide the concentration required from 0.001 to 1 mg/ml. The controls (without essential oil) were inoculated while following the same process. A mycelia disc (diameter of 5 mm) of pathogenic fungi, taken as periphery of the culture seven days backward, was inoculated aseptically in the center of the sets of Petri dish containing the treatments and control. Limp Petri was incubated with 22 ± 2°C during seven days. For each treatment, three repetitions were carried out. The diameter of the fungi colonies of the sets of treatment and diameter of positive control were measured. Mycelia inhibition of percentage was calculated by the average value of the diameters of colony by the following formula (Pandey et al., 1982).

Percentage of mycelia growth inhibition:

$$PI.\ (\%) = \left(\frac{dt - dT}{dt}\right) x100$$

Where, dt is the average diameter of the fungi colonies in the control and dT and average diameter of the treated fungi colonies.

Determination of dry weight

To determine the effect of essential oils on the dry weight of tested fungi in liquid medium, various concentrations of essential oils are prepared in a liquid medium of potato dextrose (PDB). This was done in test tubes (15 ml) and inoculated spores (10^7 spores/ml) of the fungi quads. Positive species tested in controls (without essential oils) are prepared at the same time. After a duration of the 15 days incubation at 22 ± 2°C, the mycelium is filtered through a Wattman filter paper No. 1, washed with distilled water and dried at 60°C for 6 h; and then at 40°C for a whole night. The filter paper containing the dry mycelium was weighed. The tests are repeated three times. Inhibition is calculated starting from the dry weight of the mycelium, by taking the control positive for the100% growth (Sharma and Tripathi, 2008).

Determination of the inhibiting minimal concentration (CMI)

The CMI of essential oils necessary for inhibition of the mycelia growth of tested fungi was determined by food poisoning technique (Grover and Moore, 1962). The inhibiting concentration minimal (CMI) of essential oils against the phytopathogenic fungi A was determined and the oil showed an absolute fungitoxicity. Various concentrations of the oil from 0.001 to 1 mg/ml as well as the control (without essential oils) were prepared by separately dissolving its necessary quantity in 0.5 ml 5% (v/v) tween-80 and then mixing it with 9.5 ml of medium PDA. Limp Petri inoculated was incubated during seven days at 22 ± 2°C. The weakest concentrations without observable growth (with the binocular magnifying glass) were defined like the inhibiting minimal concentration (CMI). For each concentration, three tests were carried out. The nature of toxicity (fungistatique/fungicide) A was given according to the study of Thomson (1989).

Production and germination of spores

The colonies of spore previously exposed to the oil (until the spores formed) of *F. ox, P.* sp., *A.* sp. and *F.* sp. were gathered by adding 5 ml of 0.1 ml/100 ml containing sterile distilled water and Tween-80 in each Petri dish. The suspension of spore was gathered and then centrifuged. A hemocytometer was used to count the number of spores. The percentage of inhibition of the production of spore (PIs%) was calculated relative to the control. The fungi spores are obtained starting from 10 days old fungi cultures to 6 to 10 days old *F. ox, P* sp., *A.* sp. and *F.* sp.*;* they were taken and placed on blades of glass in three specimens. The blades were incubated in an incubator at 22 ± 2°C during 48 h. For each treatment, 200 spores were examined and the germination of spores was evaluated by seeking the appearance of the tubes germinated as described by Grbic et al. (2011). The spore, whose germ tube reaches 50% of its size, is considered to be germinated. The percentage of inhibition of germination (PIg%) was calculated relative to the control as positive.

Statistical analysis

Each experiment was carried out in triples, and the average values were calculated. Statistical analysis was carried out using ANOVA and the differences between the values were given, using Duncan's test ($p < 0.05$).

RESULTS

Profile of chemical essential oils

Essential oils are extracted from the sheets of orange, Bigaradier, mandarin and lemon by hydro distillation. The yields obtained are 0.96, 1.02, 0.51 and 0.73% respectively for orange, lemon, mandarin and Bigaradier. The qualitative and quantitative analysis of the chemical profiles of essential oils is indicated in Table 1. The table includes the time of retention and percentage of the 51 components identified, accounting for 92.47 to 98.82% of all the components. The outputs obtained from the essential oils are: The results obtained proved that there are qualitative similarities between the four essential oils, although the quantities of some corresponding compounds are different. 10 componds were recorded jointly among the 51 identified, where limonene (7.18 - 36.10%), β- pinene (4.35 - 30.0%) and linalool (0.21 - 63.03%) represented the principal major compounds. The greatest volatile fraction returns of monoterpenes hydrocarbons for orange, mandarin and lemon tree are 73.64, 63.75 and 61.41%, respectively. The oxygenated monoterpenes explained approximately 71.85% of the identified components of the essential oil of Bigaradier. GC/SM of the essential oil of mandarin revealed the presence of strong concentrations in γ Terpinene (26.62%), limonene (22.52%) and β-pinene (4.35%) representing 53.49% of the total surface of the peak (Table 1). The essential oil of lemon is characterized by the prevalence of limonene (36.10%) and β-pinene (14.88%). The essential oil of orange is characterized by high concentrations in β-pinene (30%), limonene (9.37%) and the presence of two isomers sesquiterpenes (Z) and (E) β - elemene with a preponderance of the isomer (E) β - elemene (8.97%). For the essential oil of Bigaradier, linalool (63.03%) constituted the major component dominating with limonene (7.18 %) and β-pinene (5.25 %).

Antifungal activity

Inhibiting effect of essential oils on mycelia mass growth

The antifungal activity of oils essential of the Algerian citrus species with various concentrations is evaluated with respect to four phytopathogenic fungi, *F. ox, P. sp, A. sp* and *F.* sp*. in-vitro*, expressed by the inhibition percentage of the radial growth (Peak %). The results in Table 2 show the inhibiting capacity of the radial growth with a remarkable difference for the total essential oils tested. Fungi susceptibility to essential oils produces high percentages of inhibition (inhibition 100% of the mycelia growth) and has absolute toxicity. Radial growth and the biomass (Figure 1) of *F. ox, A. sp* and *F. sp* were significantly reduced in response to the various concentrations of essential oils of Bigaradier, mandarin and lemon tree, with energy of 1à 0.05 mg/ml.

***Determination of inhibiting minimal concentration* (CMI)**

More precise data on the antifungal properties of essential oils of the Algerian citrus were obtained by determining the minimal inhibiting concentrations (CMI mg/ml) as well as the nature of the fungitoxicity (Table 3). Mandarin and lemon respectively have weakest CMI 0.01 mg/ml in respect to *F. ox* and *A. sp.* 0.05 mg/ml concentration is judged to be fungicidal as follows: Bigaradier, mandarin and lemon for *F. sp;* Bigaradier and lemon *fort F. ox* and Bigaradier *for A. sp*. The fungi species *P. sp* have been shown to be more resistant to four tested essential oils. The CMI obtained was 0.1, 1.1 and > 1.0 mg/ml respectively for mandarin, lemon, Bigaradier and orange .

Inhibiting effect of essential oils on the production and germination of spores

The results of the effects of inhibition on the production and germination of spores by essential oils are represented in Figures 2 and 3. Compared with the control, they are expressed by the inhibition percentage of the production of spores (PIs %) and the inhibition percentage of the germination of spores (PIg %). Spores production was strongly inhibited by essential oils of orange, Bigaradier, mandarin and lemon in *F. ox, P. sp* and *A. sp*. The highest inhibition percentage of the production of spores was recorded in *P. sp* with lemon (97, 85%). One note a stimulation of spores production with *F. sp* for essential oils of lemon and mandarin tree.

The capacity of citrus to inhibit spores germination was

Table 1. Chemical composition of life EOs of Algerian citrus.

Component	RT (min)	Mandarin (% area)	Lemon (% area)	Orange (% area)	Bigaradier (% area)
1-hexanol	4.68			-	-
3 hexen- 1-ol	4.71	-	-	-	0.91
α-Thujene	5.81	2.00	0.21	1.49	0.02
α -Pinene	5.99	4.36	1.28	2.04	0.38
camphene	6.37	0.10	0.13	-	0.03
β - Pinene	7.12	4.35	14.88	30.0	5.25
β - Myrcene	7.46	1.19	2.56	4.52	1.40
α Phellandrene	7.86	0.10	0.21	0.68	-
3 Carene	8.02	-	0.98	6.41	0.16
(+)-4-Carene	8.20	0.38	0.34	2.77	-
Limonene	8.71	22.52	36.10	9.37	7.18
β Ocimene	8.74	-	-	0.74	-
1,3,6-octatriene,3,7-dimethyl-	9.11	0.94	3.01	8.59	1.13
γ Terpinene	9.41	26.62	1.22	4.42	-
Cis sabinene hydrate	9.77	-	-	0.21	-
Terpinolene	10.25	1.16	0.43	2.53	-
Linalool	10.71	0.21	1.40	1.24	63.03
Z Alloocimene	11.38	0.03	0.06	0.08	-
Citronellal	12.06	-	1.21	0.90	-
Terpinen-4-ol	12.94	0.15	0.74	2.14	0.43
α Terpineol	13.34	0.10	0.79	0.07	1.00
Citronellol	14.36	-	-	1.05	-
Citral	14.70	-	6.79	-	0.46
1,6-octadien-3-ol,3,7-dimethyl	14.87	0.11	1.10		5.79
2,6-octadienal,3,7-dimethyl	15.45	-	6.79	0.22	0.15
Thymol	16.97	0.30	-	-	-
σ-Elemene	17.23	-	-	-	0.05
Cis-2,6-Dimethyl-2,6octadiene	17.53	-	-	0.45	-
Benzoic acid 2-amino- methyl	17.79	0.09	-	-	-
2,6-octadien-1-ol,3,7dimethyl	17.84	-	10.49	0.22	0.99
Copaene	18.20	-	-	-	-
(Z) *β* Elemene	18.45	-	-	0.44	-
(E) *β* - Elemene	18.71	-	-	8.97	0.08
*C*aryophylene	19.41	-	1.65	3.48	2.62
Methyl N-methylanthranilate	19.74	34.02	-	-	-
Aromadendrene	19.93	-	-	-	0.05
(E) *β* Famesene	20.20		-	0.65	0.12
α -humulene	20.29	-	0.41	1.33	0.39
β -Selinene	21.13	-	-	0.47	-
γ Elemene	21.35	-	0.10	-	0.26
α -Farnesene	21.49	-	-	0.61	-
γ -cadinene	21.80	-	-	0.56	0.01
δ -Cadinene	21.95	-	-	0.22	0.08
Nerolidol	22.95	-	-	-	0. 37
*C*aryophylene oxide	23.50	0.09	0.19	0.23	0.04
α Cadinol	25.26	-	-	-	0.04
β Sinensal	26.04	-	-	0.72	-
α Sinensal	27.27	-	-	0.30	-
β Farnesol	33.89	-	0.34	-	-
Phytol	34.36	-	-	-	0.05
Monoterpene hydrocarbons (%)		63.75	61.41	73.64	15.55
Oxygenated monoterpenes (%)		34.98	28.65	6.05	71.85
Sesquiterpene hydrocarbons (%)		-	02.16	15.95	3.66
Oxygenated sesquiterpene (%)		0.09	0.53	1.25	0.45
Other **(%)**		-	-	-	0.96
Total identified (%)		98.82	92.75	96.89	92.47

RT: Retention time , - not detected.

studied based on spores from the cultures previously exposed to essential oils. Orange, Bigaradier, mandarin and lemon exposed inhibitions of the different germinated spores. The most effective one against all the tested

Table 2. Zones of growth inhibition in Percentage Inhibition (PIc %) showing antifungal activity of Citrus EOs.

Essential oil	Dose (mg/ml)	Percentage Inhibition (PIc %)[a] (mean ± SE,n=3)			
		Fusarium sp	*Fusarium oxysporum fsp albedinis*	*Alternaria sp*	*Penicellium sp*
Orange	1	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	63.92±0.3^{AB}
	0.8	100±00.0^{A}	76.84±7.8^{A}	100±00.0^{A}	60.62±4^{AB}
	0.6	100±00.0^{A}	76.59±3.5^{A}	100±00.0^{A}	56.91±10.0^{AB}
	0.4	100±00.0^{A}	65.90±6.3^{AB}	100±00.0^{A}	13.02±5.6^{C}
	0.2	88.43±0.7^{A}	50.33±8.3^{B}	73.07±4.3^{A}	N.A.
	0.1	12.35±3.3^{C}	19.40±5.5^{C}	23.33±2.6^{BC}	N.A.
	0.05	3.92±0.8^{D}	9.53±0.7^{C}	8.46±8.6^{C}	N.A.
	0.01	N.A.	2.86±0.5^{D}	5.12±1.7^{CD}	N.A.
	0.001	N.A.	N.A.	N.A.	N.A.
Bigaradier	1	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}
	0.8	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}
	0.6	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}
	0.4	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}
	0.2	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}
	0.1	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}
	0.05	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	64.92±19.3^{AB}
	0.01	N.A.	34.11±3.0^{BC}	22.25± 2.5^{BC}	18.0±5.6^{C}
	0.001	N.A.	21.50±1.02^{BC}	13.16± 3.5^{C}	N.A.
Mandarin	1	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}
	0.8	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	83.92±0.47^{A}
	0.6	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	84.51±0.21^{A}
	0.4	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	80.20±0.63^{A}
	0.2	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	74.51±0.62^{A}
	0.1	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	N.A.
	0.05	100±00.0^{A}	100±00.0^{A}	71.87±0.13^{AB}	N.A.
	0.01	73.45±0.05^{A}	100±00.0^{A}	N.A.	N.A.
	0.001	N.A.	37.93±0.27^{BC}	N.A.	N.A.
Lemon	1	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}
	0.8	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	83.14±0.20^{A}
	0.6	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	72,16±0.16^{AB}
	0.4	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	71.18±0.49^{AB}
	0.2	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	65.29±0.43^{AB}
	0.1	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	N.A.
	0.05	100±00.0^{A}	100±00.0^{A}	100±00.0^{A}	N.A.
	0.01	N.A.	30.56±0.17^{BC}	100±00.0^{A}	N.A.
	0.001	N.A.	35.25±0.10^{BC}	45.42±0.11^{B}	N.A.

[a]Values followed by same alphabetic letters are not significantly different according to ANOVA and Dunc an's Multiple Range Test (p≤ 0.05). N.A., non-active zones of growth inhibition values are presented as mean ± standard deviation.

fungi stocks was the oil of orange, with a strong inhibition percentage of spores germination of (PIg 58.66 to 100 %). A stimulation of the germination of spores was observed in *A. sp* with lemon and mandarin tree.

DISCUSSION

The bioactivity of the oils essential extracted from the sheets of Algerian citrus in respect to four phyto-

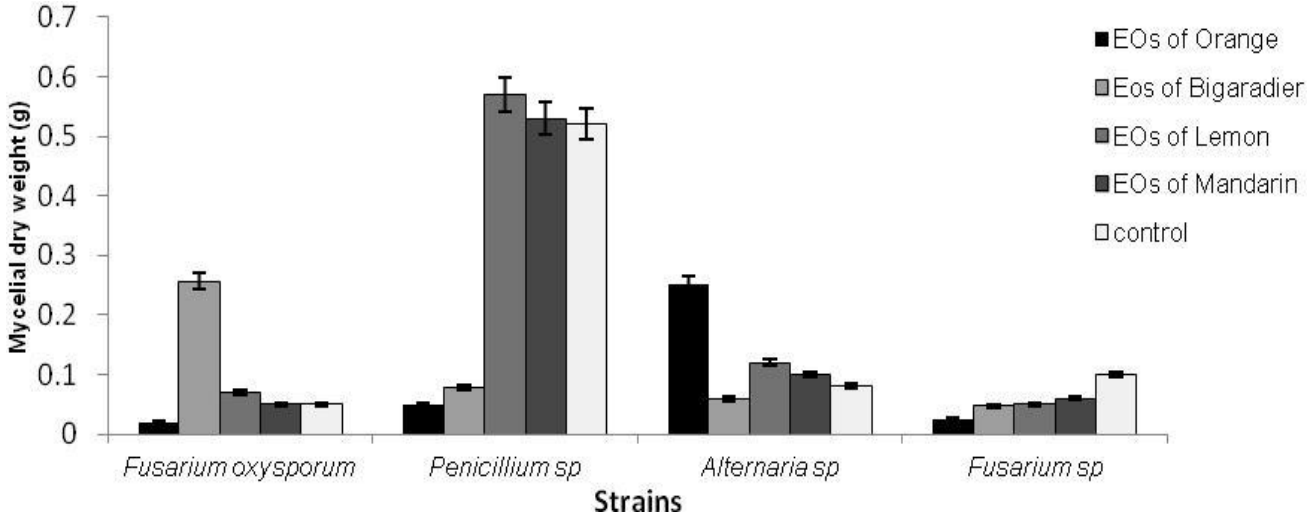

Figure 1. Effect to citrus EOs on Mycelia dry weight (g) of *fungi* after 15 day , grown in a broth of potato dextrose (PDB) and incubated at 22±2°C.

Table 3. Minimal inhibitory concentration (MIC) (mg/ml) and nature of fungitoxicity (NF) EOs of Orange, Bigaradier, Mandarin and Lemon, from (Chlef) Algeria.

Essential oil	Fungal strain							
	***Fusarium* sp.**		***Fusarium oxysporum fsp albedinis***		***Alternaria* sp.**		***Penicellium* sp.**	
	MIC (mg/m)	**NF**	**MIC (mg/ml)**	**NF**	**MIC (mg/ml)**	**NF**	**MIC (mg/ml)**	**NF**
Orange	0.4	-	1.0	+	0.4	-	>1.0	+
Bigaradier	0.05	-	0.05	-	0.05	-	0.1	-
Mandarin	0.05	-	0.01	-l	0.1	-	1	-
Lemon	0.05	-	0.05	-	0.01	-	1	+

- , Fungicidal; +, fungistatic.

pathogenic fungi was evaluated. The chemical analysis of essential oils by GC/SM indicates strong percentages in monoterpenes hydrocarbon for the whole of the oils tested except Bigaradier. Our results agree with those obtained by other authors on orange, mandarin and lemon (Baaliouamer, 1987; Espina et al., 2011; Settanni et al., 2012). The high percentage of oxygenated monoterpenes in Bigaradier was announced by Lota et al. (2001). Limonene has strong percentage in lemon tree, mandarin tree, orange and Bigaradier. Results similar to ours were given by several studies (Baaliouamer, 1987; Lota et al., 2001; Pistelli et al., 2012). The essential oil of mandarin records a strong percentage in γ Terpinene; similar results were given by Fuselli et al. (2008). With regard to the oil of orange, one noted a high percentage in β-pinene. Linalool was identified in all essential oils, but its greater quantity was announced in Bigaradier. The importance of linalool in the essential oil of Bigaradier was announced by Lota et al. (2001).

Several publications on the antifungal activity of essential oils of the citrus are reported in the literature (Viuda-Martos et al., 2008; Sharma and Tripathi, 2008). The essential oils of the Algerian citrus with proportioned concentrations demonstrated all the capacity to reduce or inhibit the growth of the fungi species tested in-*vitro.* Radial growth and biomass of *F. ox, A. sp* and *F sp* were strongly inhibited at summer in different degrees; they have a more marked activity of essential oils of Bigaradier, lemon and mandarin with very weak inhibiting minimal concentrations. Their good biological activity can be related to the presence of monoterpenes. Essential oils of the citrus were characterized by a high percentage in monoterpenes, particularly in limonene, linalool, β-pinene and γ Terpinene. Several authors allotted the antifungal activity of essential oils of citrus to the

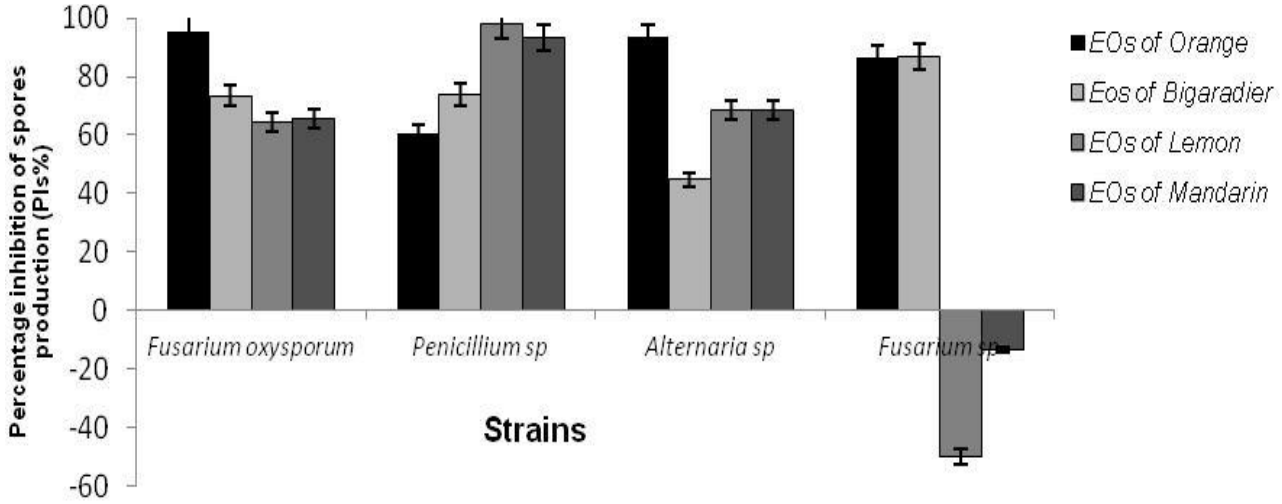

Figure 2. Percentage inhibition (compared with positive controls) of spore produced of *fungi* in colonies previously exposed to Citrus EOs. Data are means ± standard deviations (error bars).

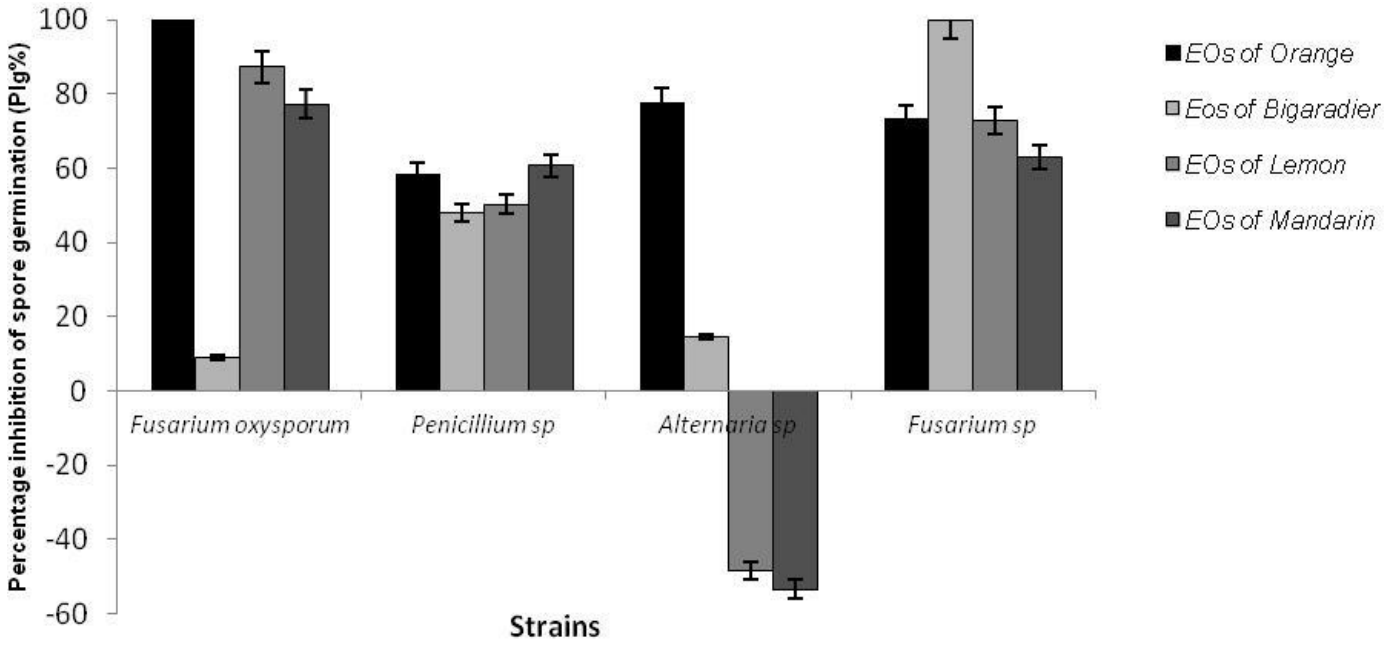

Figure 3. Percentage inhibition (compared with positive controls) of spore germination of *fungi* in colonies previously exposed to citrus Eos. Data are means ± standard deviations (error bars).

presence of monoterpenes such as linalool, limonene or central etc (Sharma and Tripathi, 2006, 2008; Viuda-Martos et al., 2008). They act on hyphas mycelia, lead to the loss of rigidity and integrity of the cellular wall of the hyphas, resulting in the death of the mycelium (Sharma and Tripathi, 2008). The capacity of citrus essential oils to inhibit the production and germination of the spores of tested fungi was evaluated *in vitro*. The tests of production and germination of spores showed the inhibiting effect of essential oils of orange, Bigaradier, mandarin and lemon on *F.* ox*, F.* sp*., P.* sp. and *A.* sp.

The capacity of citrus essential oils to inhibit the production and germination of spores was reported by several authors (Sharma and Tripathi, 2006, 2008; Chutia et al., 2009; Grbic et al., 2011). The best percentages of inhibition were presented by the oil of orange in respect to *F. ox, F.* sp. *and A.* sp. Sharma and Tripathi (2008) announce that the oil of orange was extremely toxic to

the germination of the spores of *Aspergillus niger* and that oil treatment led to distortions, thinning of the wall of the hyphas, reduction of the diameter of the hyphas and the absence of conidiophores. It is remarkable that the essential oil of the lemon stimulated the production of the spores of *F. sp* whereas the essential oil of the mandarin supported the germination of the spores of *A sp*. French (1985) recalled that it is extremely difficult to correlate the fungitoxic activity of simple compounds or classes of compounds. The various components of oil can act in a synergistic way while the association of several compounds can have a stimulating action on the germination of fungi spores.

The results obtained justify future research, stressing the antifungal properties of the major components of essential oils, on one hand, and the capacity of these oils to inhibit the fungi tested *in vivo,* on the other hand.

Conclusion

The data presented here shows the inhibiting powerful potential of essential oils extracted from the sheets species of Algerian citrus in respect to *F. ox, P. sp, A. sp and F. sp in vitro* and their possible integration in the programs of protection against the phytopathogenic fungi. The fungi anti capacities of essential oils of the Algerian citrus proved to be a very interesting field for applications in agro- alimentary industry.

Conflict of interest

The authors declare no conflict of interest.

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