Journal of Essential Oil Bearing Plants (2018)
Post-Print

Study of Antifungal, Anti-aflatoxigenic, Antioxidant Activity and Phytotoxicity of Algerian *Citrus limon* var. Eureka and *Citrus sinensis* var. Valencia Essential oils

Yamina Ben Miri [1], Agustín Ariño [2] and Djamel Djenane [1]*

[1] Laboratoire de Qualité et Sécurité des Aliments (LQSA),
University of Mouloud MAMMERI, P.O. Box 17. 15000. Tizi-Ouzou. Algeria

[2] Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA),
Veterinary Faculty, 50013 Zaragoza, Spain

*Corresponding authors (Djamel Djenane) E-mail: < djenane6@yahoo.es >

Abstract: The inhibitory influence of *Citrus limon* var. Eureka and *Citrus sinensis* var. Valencia essential oils (EOs) on the growth of *Aspergillus flavus* and AFB_1 production was evaluated. The EOs were characterized by limonene (54.95 % and 82.6 %, respectively) as predominant component. *Citrus limon* EO at 1.75 mg/mL and *Citrus sinensis* at 2 mg/mL could totally inhibit fungal growth as well as AFB_1 production. The *Citrus* EOs revealed wide spectrum of fungitoxicity against some isolated fungi in terms of MIC and MFC. On the other hand, the antioxidant activity was also assessed where IC50 and β-carotene/linoleic acid inhibition percentage of *Citrus limon* and *Citrus sinensis* oils were 1570.10 and 752.26 µg/mL, 36.19 and 55.56 %, respectively, while the total phenolic were 16.90 and 10.53 µg/mg, respectively. Additionally, the EOs showed their non phytotoxicity on wheat seeds. These findings demonstrated that EOs could be good alternatives to protect food.

Key words: *Citrus* essential oil, *Aspergillus flavus*, antifungal, aflatoxin B_1, antioxidant, phytotoxicity.

Introduction

Mycotoxins are secondary metabolites that are not necessary to fungi growth. These toxins contaminate cereals, fruits, nuts, almonds, grains, as well as foods or compounds intended for human and animal consumption [1].

The mycotoxins are secreted by fungi belonging to *Aspergillus*, *Penicillium* and *Fusarium*. Among these secondary metabolites considered important from an agriculture and health point of view, aflatoxins, which are produced by two strains of *Aspergillus*. *Aspergillus flavus* produces aflatoxins B_1 (AFB_1) and B_2 (AFB_2), while *A. parasiticus* excretes, in addition, aflatoxins G_1 (AFG_1) and G_2 (AFG_2). *A. nomius*, close to *A. flavus*, is capable of producing aflatoxins. The most toxic of the four natural aflatoxins is AFB_1. AFB_1 is metabolized by cytochrome P450 to AFB1-8, 9-epoxide that interacts with proteins and DNA inducing cell damages [2].

The development of methods for the decontamination of infested food items has become one of the major goals to ensure food safety for humans and animals. There are no specialized methods that would remove all mycotoxins. Strategies are classified according to whether they are based on chemical, physical and biological processes. They must either reduce toxins or degrade them without producing residual toxicities, developing of resistant microorganisms or decreasing the organoleptic and nutritional proprieties of food.

A new approach has been developed by using

natural products to decrease the fungal growth and mycotoxin contamination in food [3]. Essential oils (EOs) produced by such plants as *Carum carvi* L., *Thymus vulgaris*, Citrus *aurantifolia* and *Cinnamomum zeylanicum* L. EOs have been supported because of their antimicrobial activity [4-5]. EOs are liquids obtained by hydro-distillation method from different parts of plant and composed of variety of bioactive components that is why they are interesting in antimicrobial activity. The application of EOs is in demand by consumer to preserve foods because they are generally recognized as safe (GRAS) [6].

The present study investigated the antifungal, antiaflatoxin, total phenolic content, antioxidant activity and phytotoxicity of *Citrus limon* var. Eureka (lemon) and *Citrus sinensis* var. Valencia (orange) EOs.

Materials and methods

Chemicals and equipment

Chemical products and solvents used in the study were obtained from different companies in different countries: chloroform, methanol, dimethyl sulfoxide (DMSO), isoamyl alcohol, hexane, anhydrous sodium sulphate (Na_2SO_4), linoleic acid, Folin-Ciocalteu, Na_2CO_3, gallic acid, butylated hydroxytoluene (BHT), Potato Dextrose Agar (PDA) medium (Potato, 200 g; Dextrose, 20 g; Agar, 15 g and distilled water 1000 mL), Malt Extract Agar (M.E.A) (Malt extract, 20 g; Peptone, 1 g; Glucose, 20 g; Agar, 20 g; Distilled water, 1000 mL), Czapek concentrate (N_aNO_3, 30 g; KCl, 5 g; $MgSO_4.7H_2O$, 5 g; $FeSO_4.7H_2O$, 0.1 g; Distilled water, 1000 mL), Glycerol Nitrate Agar (G25N) (K_2HPO_4, 0.75 g; Czapek concentrate, 7.5 mL; Yeast extract, 3.7 g; Glycerol, 250 g; Agar, 12 g; Distilled water 750 mL), *Aspergillus flavus* and *parasiticus* agar (AFPA) (Peptone, 10 g; Yeast extract, 20 g; Ferric ammonium citrate, 0.5 g; Chloramphenicol, 100 mg; Agar, 15 g; Dichloran, 2 mg; Distilled water, 1000 mL), SMKY medium; (Sucrose, 200 g; $MgSO_4.7H_2O$, 0.5 g; KNO_3, 0.3 g and yeast extract, 7 g; 1000 ml distilled water) were obtained from Sigma-Aldrich (France). Tween 40 and tween-80 from Sigma-Aldrich (Germany). 2,2-diphenyl-1-picrylhydrazil (DPPH) and β-carotene were purchased from Sigma-Aldrich (Netherland). Silica gel-G 60 from Fluka (Germany).

Hydro-distillation apparatus, GC-MS (Agilent Technologies; model 6850 and 5973, United Kingdom), centrifugation apparatus (Jouan E76, United Kingdom), UV lamp (CN-6, Vilber Lourmay, France), spectrophotometer (6705 UV/Vis, Jenway, United Kingdom) and a light microscope (Motic: BA210, China) were used in the present investigation.

Plant material

The fruits of *C. limon* var. Eureka and *C. sinensis* var. Valencia were harvested from the experimental field of the Institute of fruit-farming technology, in Mitidja province, in Algeria, in March and April 2015. Botanical authentication of the species was given by the same Institute.

Extraction of essential oils

EOs were extracted from *C. limon* and *C. sinensis* peels (200 g) by hydrodistillation at atmospheric pressure, using a Clevenger type apparatus for 4 h. The obtained EOs were dried with anhydrous sodium sulphate (Na_2SO_4) and then recovered, weighed and stored in brown vials, hermetically sealed and stored at 4°C before being used.

Essential oil analysis

Gas chromatography-Mass spectrometry (GC-MS) analysis

The chemical composition of the EO was analyzed using GC-MS. The EO (10 µL) was dissolved in hexane (100 µL) and 2 µL of the solution was injected into a GC-MS (Agilent; model 6850 and 7890). The capillary column was DB-5 (length = 30 m × 0.25 mm i.d., film thickness = 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The column inlet pressure was 8.07 psi. The GC column oven temperature was increased from 60 to 245°C at 3°C/min, with a final hold time of 4 min. The Electron Ionization-Mass Spectrometry (EI-MS) operating parameters were as follows: electron energy, 70 eV; automatic scanning of the mass range 50-550 amu; ion source temperature, 230°C; quadrupole, 150°C.

The identification of the volatile compounds was done by comparing the mass spectra (MS) ob-

tained with the NIST electronic databases, as well as with the bibliography [7] in parallel with the use of retention indices (IR) based on series of *n*-alkane indices (C8-C28) on the capillary column.

Fungal material and confirmation of testing strain

A. flavus E73 was obtained from Laboratoire de Biologie des Systmes Microbiens (LBSM; Kouba; Algeirs; Algeria). Confirmation of strain was realized according to the morphological characteristics of the mycelium, by microscopic observation, and single spore method after their culture on Malt Extract Agar (M.E.A), Glycerol Nitrate Agar (G25N) and Czapek Yeast Agar (C.Y.A), the confirmation was also carried out by inoculation in *A. flavus* and *parasiticus* agar (AFAP) medium. This medium confirms that it belongs to the group *A. flavus*. In this medium the color of the reverse side of the colony is yellow-orange [8]. Afterwards, *A. flavus* E73 was maintained on Potato Dextrose Agar (PDA) at 4°C. Some other fungi *viz. A. carbonarius*, *A. fumigatus*, *A. niger, A. ochraceus*, *A. tamarii*, *A. terreus*, *Fusarium* sp., *Penicillium* sp., *Rhizopus* sp., isolated during mycological analysis of some edibles (spices and cereals) in our labora-tory in order their fungitoxicity spectrum.

Test confirming the aflatoxinogenicity of the strain

The method consisted in cultivating the *A. flavus* E73 (disc of 6 mm diameter) strain in Erlenmeyer flask containing 25 mL of the Sucrose Potassium nitrate Magnesium sulfate Yeast (SMKY) liquid medium for 10-day incubation period at 28 ± 2°C. The content was filtered (Whatman no.1) and extracted with 20 mL chloroform. After stirring and then decanting, the chloroform phase was recovered, evaporated and redissolved in 1 mL chloroform. A volume of 50 µL of sample was spotted on a thin layer chromatography (TLC). The development of the chromatograms was car-ried out in a standard tank (20 × 20 cm) previ-ously saturated with the solvent system: toluene: iso-amyl alcohol: methanol (90: 32: 2; v / v / v). After migration, the plates was removed and dried at 60°C for 24 h. AFB1 were detected by placing the plate in UV transilluminator (360 nm). The AFB1 appeared as a blue spot. The intensity of the fluorescence of the spots confirmed the pres-ence of AFB1 [9].

Preparation of fungal spore suspension

The spores of *A. flavus* E73 grown on PDA medium (culture of 7 days) were recovered by washing the Petri dish with a volume of 20 mL of 0.1% tween 80 sterile solution. The suspension was then filtered through sterile muslin tissue. Spore concentration (1×10^6 spores/mL) was determined by Mallassez cell (depth 0.2 mm, 1/400 mm^2) under a light microscope. The number of spores of 1×10^6/mL was fixed throughout our study.

***In vitro* antifungal activity of *C. limon* and *C. sinensis* EOs**

The evaluation of the activity of *C. limon* and *C. sinensis* EOs on the mycelial growth of *A. flavus* E73 was carried out using the direct contact technique. The method of the poisoned medium used to test the sensitivity of the fungal strain to the different EOs was that of Singh [10] and José Velázquez-Nuñez *et al.* [11]. EOs were added to 10 mL PDA and mixed at 45-50°C in Petri dishes to obtain final concentrations ranging from 0.25 to 2 mg/mL selected after preliminary tests. After solidification of the medium in each Petri dish, 10 µL of a suspension of 1×10^6 spores/mL was deposited in the center of each Petri dish. The control plates were prepared in parallel without EO. The plates were incubated at 28±2°C. Three repetition of each treatment were carried out. The mycelial growth was followed by measuring the diameter of two straight lines perpendicular to the center. Measurements were made every day for 7 days. The comparison of the dimensions obtained with those of the controls made it possible to calculate the percentage inhibition (I %) at day 7, according to the following formula:

$$I\ \% = \left(1 - \frac{Da}{Db}\right) \times 100$$

Where Da: the diameter of the zone of growth of the test in cm; Db: the diameter of the growth zone of the control in cm.

Determination of minimum inhibitory concentration (MIC and minimum fungicidal concentration (MFC

The MIC and MFC for *A. flavus* E73 were determined by broth dilution using the method of Shukla *et al.*[12]. Different concentrations of *C. limon* and *C. sinensis* EOs (0.25 to 2 mg/mL) were added to 10 mL SMKY medium in test tubes. Tubes with only SMKY medium used as control. The tubes were inoculated with spore suspension and incubated at 28 ± 2°C for 7 days. The lowest concentration of EOs capable of inhibiting the growth in test tubes during 7 days was considered as the MIC. After 7 days, the test tubes where the inhibition was total subcultured in Petri dishes containing fresh PDA. When there was a resumption of mycelial growth, the concentration was considered as the MFC.

Anti-aflatoxin test of *C. limon* and *C. sinensis* EOs

According to Mishra *et al*.[9], suspensions (50 µL) of *A. flavus* E73 were inoculated in 25 mL of SMKY medium supplemented with different concentrations of each EO. Cultures were incubated at 28 ± 2°C. SMKY broth containing only 50 µL of spore suspension as a control. Three repetition of each treatment were done. For the extrac-tion of AFB_1, the same procedure was followed as described in section of test confirming the aflatoxinogenicity of the strain. The mycelia pro-duced in the liquid cultures were removed and washed on Whatman No. 1 filter paper. The weight of the mycelium was determined after desiccation at 80°C for 12 h. For the quantifica-tion of AFB_1, the blue spots on TLC plates were scraped out, dissolved in 5 mL cold methanol and centrifuged at 2000 g for 5 min. The absorbance of the supernatant was made using a UV-Visible spectrophotometer at 360 nm. The quantity of AFB_1 was calculated according to the formula by Tian *et al*. [13]:

AFB_1 content µg/mL = (D x M) / (E x *l*) x 1000

Where D is the absorbance, M is the molecular weight of AFB_1 (312 g/mol), E is the molar extinc-tion coefficient (21, 800 l/mol.cm), and l is the path length (1 cm cell was used).

AFB_1 inhibition was also calculated as follows:

I % = (1 X/Y) x 100

Where X (µg/mL) is the mean concentration of AFB_1 in the treatment and Y (µg/mL) is the mean concentration of AFB_1 in the control.

Spectrum of fungitoxicity of *C. limon* and *C. sinensis* EOs

The MIC and MFC of EOs against some isolated fungi *viz. A. carbonarius*, *A. fumigatus*, *A. niger, A. ochraceus*, *A. tamarii*, *A. terreus*, *Fusarium* sp., *Penicillium* sp., *Rhizopus* sp., was evaluated by using SMKY medium at the selected concentrations (0.5 to 2 mg/mL) as described before to record their fungitoxic spectrum.

Determination of total phenolic content of EOs

As reported by Dewanto *et al.*[14], 125 µL of EOs in DMSO was dissolved in 500 µL of distilled wa-ter and 125 µL of Folin-Ciocalteu reagent 10 times diluted. The mixture was agitated and incubated for 3 min, and then 1.25 mL of 7 % Na_2CO_3 was added, adjusting with distilled water to 3 mL. Af-ter incubation for 2 h at 25 ± 2°C, the absorbance at 760 nm was measured. The same procedure was also applied to the standard solutions of gal-lic acid (25-200 µg/mL). The concentration of total phenolic contents of the oils was calculated from the regression equation of the curve established with the standard gallic acid and expressed in micrograms of equivalents, gallic acid per milli-gram of EO. All tests were carried out in tripli-cate.

Antioxidant activity

DPPH free radical scavenging assay

The test evaluates the capacity of the EO to scavenge 2, 2-diphenyl 1-picrylhydrazil radical (DPPH). Briefly, in clean and dry tubes, volumes of 50 µL of different concentrations (100, 200, 400, 600, 800 and 1000 µg/mL) of each EO and standard BHT were added to 5 mL of 0.004 %(w/v) methanolic solution of DPPH and incubated in darkness at room temperature for 30 min. Thereafter their absorbances were measured against a blank at 517 nm by spectrophotometer

[15]. DPPH radical-scavenging activity was expressed in terms of inhibition percentage (I %) and was calculated using the following formula :

$$I\ \% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

A_{blank} is the absorbance of the control, and A_{sample} is the absorbance of the sample.

The value of the inhibitory concentration (IC_{50}) represents the dose of the EO which causes the neutralization of 50 % of the DPPH radicals. IC_{50} was estimated by extrapolation by plotting the percent inhibition (I %) versus concentration curves. All tests were performed in triplicate.

Beta-carotene/linoleic acid bleaching assay

This complementary method is used to assess the antioxidant activity of compounds. As described by Miraliakbari and Shahidi [16], 0.5 mg of β-carotene was dissolved in 1 mL of chloroform, 25 µL of linoleic acid and 200 mg Tween 40. The chloroform was totally evaporated; then 100 mL of aerated distilled water was added and the mixture was shaken. The samples (2 g/L) were dissolved in DMSO and 350 µL of each sample solution were added to 2.5 mL of the resulted mixture and incubated in water bath at 50°C; for 2 h with controls. BHT was used as a positive control and DMSO as a negative control. The absorbance was measured at 470 nm by spectrophotometer and the antioxidant activities (I %) was calculated using the following formula:

$$I\ \% = (A_{\beta\text{-Carotene after 2 h assay}} / A_{\text{initial }\beta\text{-Carotene}}) \times 100$$

Where, $A_{\beta\text{-Carotene after 2 h assay}}$ is the absorbance of β-Carotene after 2 h assay and $A_{\text{initial }\beta\text{-Carotene}}$ is the absorbance of β-Carotene at the beginning of the experiments. All tests were carried out in triplicate.

Phytotoxicity assay of *C. limon* and *C. sinensis* EOs

The phytotoxicity of EOs was determined for varieties of *Triticum aestivum* (wheat) *viz*. AS 81189 A (Ain Abid) and HD 1220 (Hiddab) (Dar El Beida, Algiers, Algeria) following the method proposed by Kordali *et al*.[17]. After surface-sterilising of wheat seeds with sodium hypochlorite (1 %) for 20 min, rinsing and removing empty and undeveloped seeds. Two layers of filter paper were placed on each Petri plate and then 10 mL of distilled water were poured. Afterwards, 50 wheat seeds were deposited on the filter paper. Ten microliters (10 µL) of each EO was dropped on Whatman no.1 and placed on the lid. Controls were also prepared but no EO was added. Petri plates were closed with parafilm and incubated at 23 ± 2°C. After 8 days of incubation, the experiment was stopped and the percentage of germination of each variety was determined. The germination rate corresponds to the maximum percentage of germinated seeds in relation to the total seed number. After determining the number of seeds that germinated, the lengths of the radicle and the plumule were measured. Triplicates were carried out for each variety.

Statistical analysis

All data are reported as means ± standard deviations (SD). The significant differences between mean values were determined by Newman and Keuls multiple range test ($p \leq 0.05$), following one-way ANOVA. The statistical analysis was performed using Statbox 6.40. T-test was used to determine the significance of difference between the phytotoxicity experiments and the control by STATISTICA version 6. $P < 0.05$ were taken to be statistically significant.

Results and discussion

Yield and chemical composition of EOs

C. limon and *C. sinensis* EOs provided a level of about 0.62 ± 0.13 and 0.55 ± 0.05 %, respectively. Data were in accordance with the findings of Djenane [18] who observed that the yield of *C. limon* and *C. sinensis* EOs was 0.70 % and 0.58 %, respectively. However, Tue *et al*.[19] reported that the yield of *Citrus* EOs was differing with individual plant species ranging from 0.2 to 2.0 %. Ahmad *et al.* [20] reported that *C. limon* EO yield was 1.12 %. Sharma and Tripathi [21] found that *C. sinensis* EOs yielded 1.8 %.

The constituents identified by GC-MS analysis, their retention indices and relative proportions are summarised in Table 1. In the present investigation, the GC-MS analysis showed that

Table 1. Chemical composition of *C. sinensis* and *C. limon* EOs

Components[a]	RI_E	RI_L	% *C. sinensis*	% *C. limon*
trans-2-Hexenal	833	846	tr	-
α-Thujene	918	924	tr	0.19
α-Pinene	926	932	0.43	0.85
Camphene	943	946	-	tr
Sabinene	965	969	0.38	0.77
β-Pinene	971	974	0.30	5.90
Methylheptenone	975	980 [48]	-	tr
Myrcene	981	988	1.56	1.07
Dehydro-1,8-cineole	983	988	-	tr
n-Octanal	998	998	0.85	0.14
α-Phellandrene	1002	1002	tr	tr
δ-3-Carene	1004	1008	0.06	-
α-Terpipene	1011	1014	0.06	0.32
p-Cymene	1020	1020	-	0.91
Limonene	1026	1024	82.6	54.95
trans-β-Ocimene	1038	1044	0.07	0.10
γ-Terpinene	1051	1054	0.60	7.25
n-Octanol	1065	1063	0.08	0.09
p-Mentha-3,8-diene	1074	1068	tr	-
Terpinolene	1079	1086	0.11	0.71
p-Cymenene	1085	1089	-	tr
Linalool	1095	1095	4.99	1.45
n-Nonanal	1099	1100	0.26	0.23
1,3,8-p-Menthatriene	1108	1108	-	0.05
Methyloctanoate	1116	1116 [49]	0.08	-
cis-p-Menth-2-en-1-ol	1119	1118	tr	0.07
cis-Limoneneoxide	1131	1132	0.12	0.06
cis-p-Menth-2,8-dien-1-ol	1134	1133	-	tr
neo-allo-Ocimene	1137	1140	tr	0.08
Camphor	1141	1141	-	0.05
1,4-Dimethyl-γ-3-tetrahydroacetophenone	1143	1145 [50]	-	0.07
Isopulegol	1143	1145	tr	-
Citronellal	1145	1148	0.29	0.33
Isoneral	1154	1160	-	0.07
Borneol	1167	1165	-	0.08
Terpinene-4-ol	1175	1174	0.49	1.49
Naphthalene	1179	1178	tr	tr
p-Cymene-8-ol	1183	1179	-	0.05
n-Butyl n-hexanoate	1185	1186	tr	-
α-Terpineol	1191	1186	1.09	1.88
cis-Piperitol	1195	1195	tr	tr
n-Decanal	1201	1201	0.63	0.09
trans-Piperitol	1205	1207	-	0.05

table 1. (continued).

Components[a]	RI_E	RI_L	% *C. sinensis*	% *C. limon*
trans-Carveol	1216	1215	0.09	0.09
Citronellol	1224	1223	0.37	0.94
Nerol	1220	1227	0.16	1.61
Neral	1232	1235	0.63	2.78
Carvone	1239	1239	0.10	0.19
Geraniol	1248	1249	0.15	1.45
Methylcitronellate	1257	1257	tr	tr
Geranial	1262	1264	0.78	3.63
Perillaldehyde	1269	1269	0.27	0.23
o-Acetanisole	1287	1290	-	tr
Carvacrolethylether	1297	1297	0.06	0.06
m-Acetylanisole	1301	1298	-	tr
Carvacrol	1305	1298	-	tr
p-Vinyl-guaiacol	1309	1309	tr	-
Methylgeranate	1315	1316[51]	-	tr
Methylcaprinate	1318	1325[52]	tr	-
Citronellylacetate	1342	1350	-	0.19
Nerylacetate	1353	1359	tr	1.60
α-Copaene	1367	1374	tr	-
Geranylacetate	1371	1379	-	0.78
Ethylcaprate	1389	1380[53]	tr	-
cis-α-Bergamotene	1404	1411	-	0.11
β-Caryophyllene	1410	1417	0.05	0.74
β-Copaene	1421	1430	0.05	-
trans-α-Bergamotene	1428	1432	tr	1.12
Geranylpropionate	1441	1444[54]	-	tr
α-Humulene	1447	1452	0.05	0.15
β-Santalene	1451	1257	-	0.06
β-Acoradiene	1469	1469	-	tr
trans-β-Farnesene	1475	1471[55]	-	0.08
Valencene	1484	1496	0.15	0.20
Bicyclogermacrene	1486	1500	-	0.32
α-Muurolene	1491	1500	tr	-
E,E-α-Farnesene	1498	1505	tr	0.18
β-Bisabolene	1501	1505	tr	2.14
δ-Cadinene	1510	1522	0.08	0.09
β-Sesquiphellandrene	1519	1521	tr	tr
cis-γ-Bisabolene	1533	1529	-	tr
Elemol	1543	1548	tr	-
trans-Sesquisabinene hydrate	1578	1577	-	tr
γ-Eudesmol	1626	1630	tr	tr
t-Muurolol	1638	1640	-	tr
α-Cadinol	1650	1652	0.02	0.05

table 1. (continued).

Components[a]	RI_E	RI_L	% *C. sinensis*	% *C. limon*
β-Bisabolol	1664	1674	-	0.06
α-Bisabolol	1683	1685	-	0.27
β-Sinensal	1686	1699	0.36	-
α-Sinensal	1743	1755	0.11	-
Total identified			99.10	99.11
Monoterpene Hydrocarbons			86.25	73.27
Oxygen-containing Monoterpenes			9.73	19.34
Sesquiterpene Hydrocarbons			0.54	5.3
Oxygen-containing Sesquiterpenes			0.55	0.44
Others			2.03	0.72

[a]Components listed in order of elution on DB-5 column.
RI_E- experimentally determined retention indices on the mentioned column by co-injection of a homologous series of *n*-alkanes C8-C28.
RI_L- literature retention indices (7, 48, 49, 50, 51, 52, 53, 54, 55). tr- trace (< 0.05%)

different group of terpenoid compounds was present. In *C. limon* EO, the monoterpene hydrocarbons are mainly represented by limonene (54.95 %) and the oxygenated monoterpenes were represented by geranial (3.63 %), neral (2.78 %), α-terpineol (1.88 %), nerol (1.61% %), neryl acetate (1.60 %), terpene-4-ol (1.49 %), geraniol (1.45 %) and linalool (1.45 %). However, the sesquiterpene hydrocarbons and oxygenated sesquiterpenes were minor. *C. sinensis* EO consisted of monoterpene hydrocarbons where the predominant component was limonene (82.6 %). The main oxygenated monoterpenes were linalool (4.99 %), α-terpineol (1.08 %). The sesquiterpene hydrocarbons and oxygenated sesquiterpenes were in fewer quantities.

These data were somehow similar to the findings of Sharma and Tripathi [21], where limonene (84.2 %) and linalool (4.4 %) were the major component in *C.sinensis* (L.) Osbeck EO composition. Djenane [18] studied *C. sinensis* and *C. limon* peels EOs from Algeria. According to him the main constituent in *C. sinensis* EO were limonene (77.37 %) and β-pinene (3.45 %), whereas, major constituents in *C. limon* EO were limonene (51.39 %), β-pinene (17.04 %) and γ-terpinene (13.46 %). *C. maxima* Burm and *C. sinensis* (L.) Osbeck EOs were also characterized by limonene (31.83 % and 90.66 %, respectively) as predominant component. As well, *C.maxima* EO was consisted by other constituents such as E-citral (17.75 %), 1-hexene-4-methyl (15.22 %) and Z-citral (13.38 %) [22].

This difference among the citrus EOs yield and chemical composition related to variety and age of plant, environmental conditions, harvesting time and extraction method.

Identification of the fungal strain

Data of the identification exhibited that the fungus used for the present investigation belonged to *A. flavus* because it showed different colors on the used culture media, which facilitated its identification according to the identification key. Besides, the test of aflatoxinogenicity showed that *A.flavus* E73 synthesized AFB_1.

Antifungal activity assay

The antifungal activity of *C. limon* and *C. sinensis* EOs for the seven days is presented in Figure 1. Fungal growth was significantly ($p \leq 0.05$) diminished with increasing concentration of EOs, which indicated dose dependent activity. The percentage inhibition of fungal growth was determined at day 7 (Figure 2). *C. limon* EO caused 75.14 % inhibition in fungal growth at 1.5 mg/mL

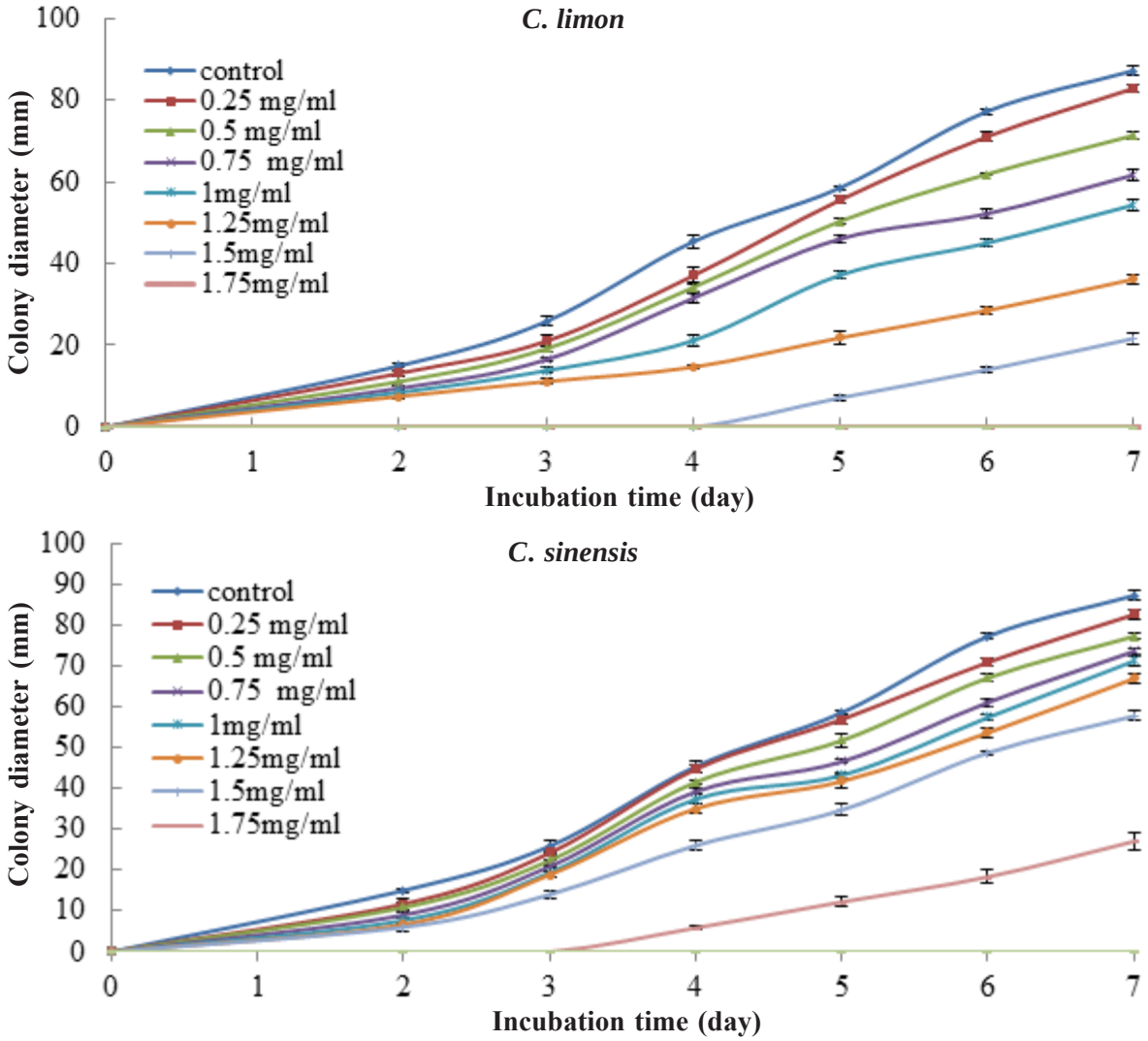

Figure 1. Influence of different concentrations of *C. limon* and *C. sinensis* EOs on *A. flavus* E73 growth during 7 days

compared with the control ($p \leq 0.05$). *C. limon* EO showed complete inhibition of *A. flavus* E73 at 1.75 mg/mL and *C. sinensis* EO provided 68.24 % inhibition at the same concentration compared with the control ($p \leq 0.05$).

Despite the low proportion of limonene (54.95 %) in *C. limon* EO when compared to that of C. *sinensis* (82.6 %), it showed high antifungal activity against *A. flavus* E73, but it might not be related to this constituent. It has been known that the antifungal activity of this EO was affected by γ-terpinene, p-cymene [23-24] and pinenes, which have been shown to have good antifungal activity [25]. Thus, these components could play a significant role in the pronounced activity of *C. limon* EO. Moreover, the antifungal activity of this EO can be attributed to citronellol, geraniol, α-terpineol and nerol. Linalool and terpinen-4-ol exhibited also good activity [26]. Monoterpene alcohols increase the permeability of the plasma membrane and inhibit process of respiration on mitochondrial membrane of fungi [27-28]. However, the antifungal activity of EOs cannot be related to single components because they are constituted by variety of volatile compounds. It is the result of synergistic and antagonistic action of the major and minor constituents of EOs [29]. Therefore, the chances of resistance development in fungi after application of the EO would be reduced, and the sensitivity to its action would be wider. Moreover, the puri-

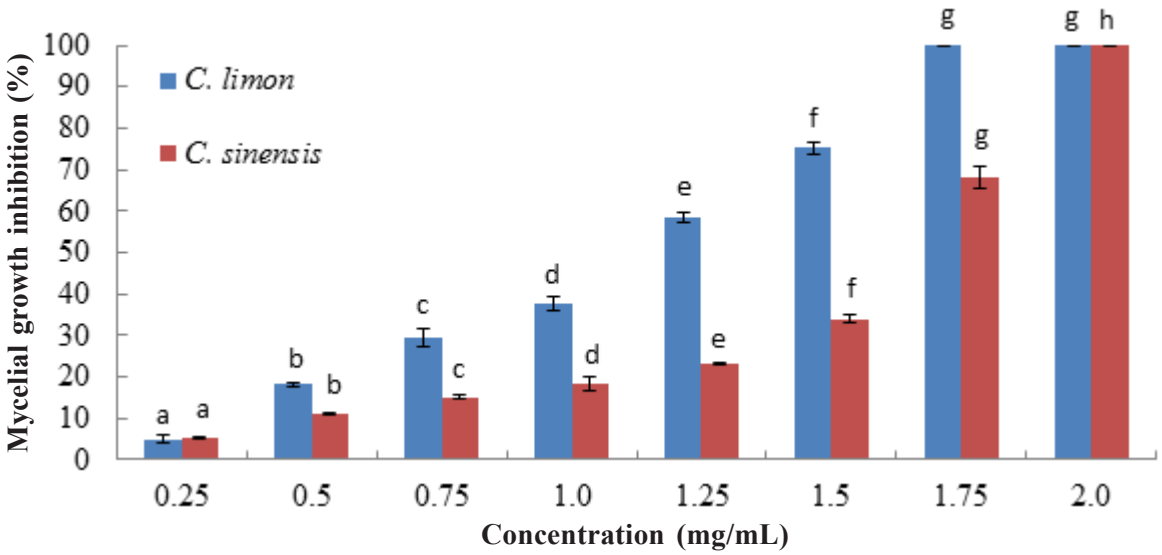

Figure 2. Percentage inhibition of *A.flavus* E73 growth after 7-day. Columns with the different letters are significantly different ($p \leq 0.05$)

fication of the individual compounds would require higher costs, so whole EOs appeared to be more promising in commercial application than single compounds.

The study of MIC and MFC is important to determine the minimum dose to control fungal populations [30]. The MIC/MFC test has been assessed against *A. flavus* E73 by the broth dilution method using SMKY medium. Kalemba and Kunicka [31] reported that the method gives opportunity to EOs to come in close contact with fungal spores in the medium. The MIC against *A. flavus* E73 was recorded at 1.75 mg/mL for *C. limon* and 2 mg/mL for *C. sinensis*. The EOs of *C. limon* and *C. sinensis* at these two concentrations, completely inhibited the growth of the fungal strain. The MFC of *C. limon* and *C. sinensis* occurred at 2 mg/mL and 2 mg/mL, respectively.

Data showed that the MIC of the used EOs in the present study was lower than *C. sinensis* var. Valencia [11] and were higher than the *C. maxima* Burm. and *C. sinensis* (L.) Osbeck [22]. It should be noted that the variation in MIC of EOs can be explained by the difference in the chemical con-stituents, the test fungus and the medium chosen for evaluating of antifungal activity.

Efficacy of the EOs on dry mycelium weight and AFB_1 content

The effect of the EOs of *C. limon* and *C. sinensis* on mycelium dry weight and AFB_1 production ($p \leq 0.05$) is presented in Figure 3. It can be clearly seen that *C. limon* EO showed marked inhibition of mycelium dry weight and AFB_1 at all concentrations where complete inhibition was observed at 1.75 mg/mL. While, for EO of *C. sinensis*, the complete inhibition was observed at 2 mg/mL.

C. limon and *C. sinensis* EOs can inhibit mycelium dry weight and the AFB_1 production. A direct correlation between fungal growth and AFB_1 synthesis has been observed. Reddy *et al.* [32] revealed that *Syzigium aromaticum* EO at 5 g/kg inhibited *A. flavus* growth and AFB_1 production. Whereas, *Caesulia axilaris* Roxb. EO showed quite inhibition of the *A. flavus* growth at 1.0 µL/mL and AFB_1 production at 0.8 µL/mL [33]. Furthermore, Vilela *et al.*[34] indicated that inhibition of AFB_1 produced by *A. flavus* and *A. parasiticus* with *Eucalyptus globulus* EO needed higher concentration than that for fungal growth inhibition. From the findings presented by these authors, it is clear that the inhibition of AFB_1 cannot be totally attributed to fungal growth inhibition. The interference with some key enzymes of carbohydrate catabolism pathway in *A. flavus*, decreased its potential to produce AFB_1 [13]. Thereby, *C. limon* and *C. sinensis* EOs could act in the same manner to control the synthesis of AFB_1 by the *A. flavus* E73. Further research is required in

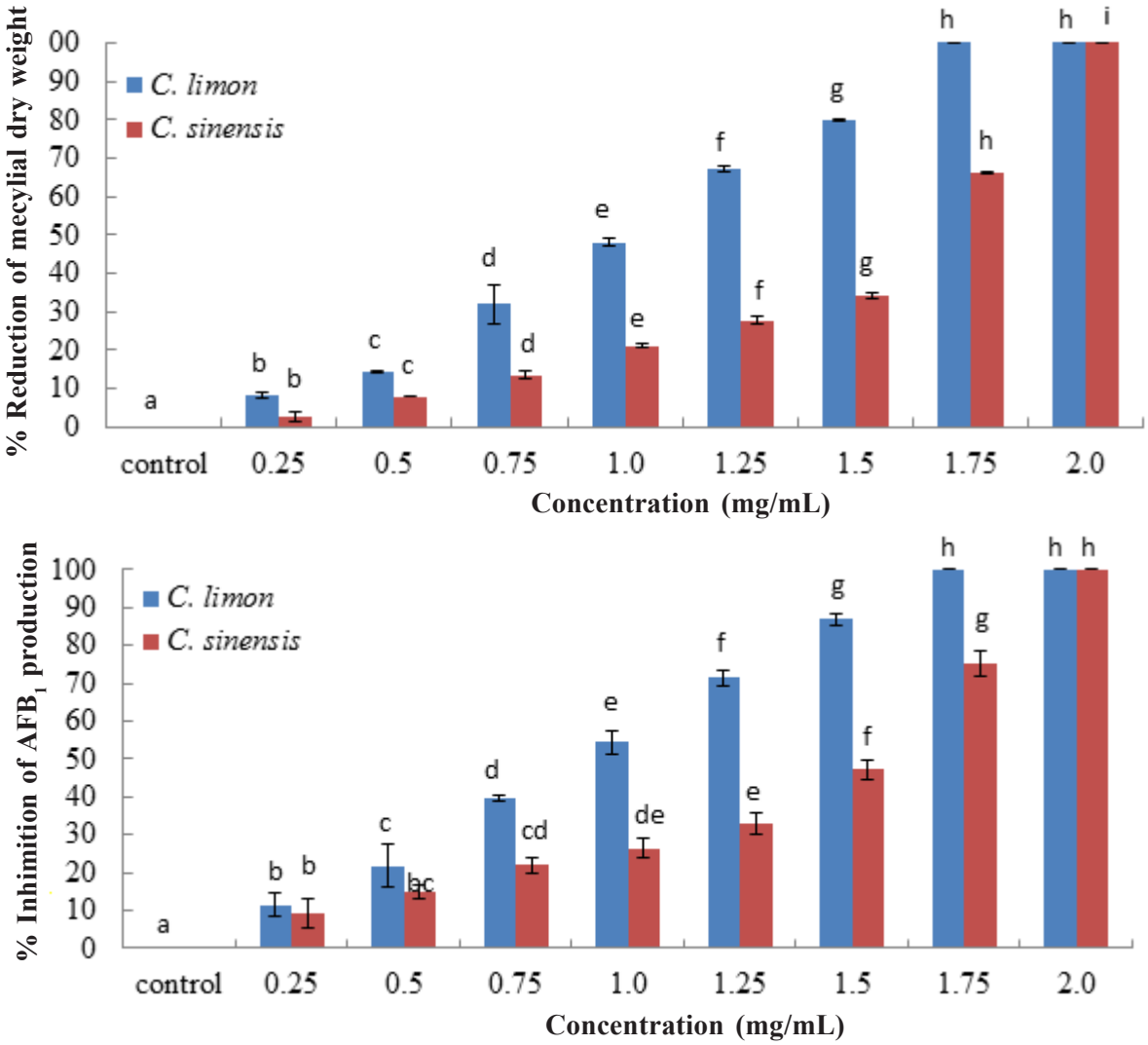

Figure 3. Effect of EOs on mycelia dry weight and AFB_1 production by *A. flavus* E73. Columns with the same letters are not significantly different. Values are mean (n = 3) ± SD

order to understand mechanisms action for the control of AFB_1 production because the majority of previous works focus more on the detection of EOs' antifungal and antiaflatoxin activity, hence, the mechanisms of action are poorly studied, most of which are assumptions.

Spectrum of fungitoxicity

The spectrum of fungitoxicity at different concentrations against the isolated fungi has been also investigated. Data of the activity of *C. limon* and *C. sinensis* EOs are presented in Table 2. They showed complete inhibition of growth of all the fungi studied. *C. limon* EO was more effective, which inhibited the growth of most fungi. *Fusarium* sp. and *Rhizopus* sp. (1.75 mg/mL) necessitated the highest concentration of this EO for inhibition and the lowest was for *A. terreus* (0.75 mg/mL). Results indicated that *C. limon* EO showed lower inhibitory concentration compared to *C. sinensis* EO except *A. niger* and *Fusarium* sp., where the inhibition was the same (1.5 and 1.75 mg/mL, respectively). *A. ochraceus* was in-hibited at 2 mg/mL of *C. sinensis* EO, which made it the most resistant to this EO compared to the other tested fungi. As well, MFC was determined for *C. limon* and *C. sinensis* EOs. It should be noted that MFC values were higher than these of MIC, they were between 1 and > 2 mg/mL for both EOs.

Table 2. Spectrum of fungitoxicity of *C. sinensis* and *C. limon* EOs

Fungi	CMI (mg/mL)		CMF (mg/mL)	
	C. limon	*C. sinensis*	*C. limon*	*C. sinensis*
A. carbonarius	1.0±0.00	1.75±0.25	1.25±0.5	>2.0
A. fumigatus	1.0±0.43	1.5±0.00	1.5±0.25	1.75±0.00
A. niger	1.5±0.00	1.5±0.14	1.75±0.00	2.0±0.00
A. ochraceus	1.5±0.00	2.0±0.00	2.0±0.00	>2.0
A. tamarii	1.25±0.25	1.5±0.72	1.75±0.00	1.75±0.00
A. terreus	0.75±0.25	1.75±0.00	1.0±0.25	1.75±0.43
Fusarium sp.	1.75±0.00	1.75±0.00	2±0.00	>2.0
Penicillium sp.	1.5±0.43	1.75±0.00	1.75±0.00	>2.0
Rhizopus sp.	1.75±0.00	2.00±0.00	>2.0	>2.0

Values are given as means of three repetitions

A lot of research has investigated the fungitoxicity potential of *Citrus* EOs. Sharma and Tripathi [21] in their work reported that *C. sinensis* (L.) Osbeck EO was found to be fungicidal against *A. niger*. Viuda-Martos *et al*.[35], reported that the EOs of lemon, orange, mandarin and grapefruit peels showed, the potential to inhibit the growth of *Penicillium chrysogenum*, *Penicillium verrucosum*, *A. niger* and *A. flavus*. Singh *et al*.[22], in their work reported that *C. maxima* Burm. and *C. sinensis* (L.) Osbeck EOs was found to be fungicidal against *A. fumigatus*, *A. niger*, *A. terreus*, *Alternaria alternata*, *Cladosporium herbarum*, *Curvularia lunata*, *Fusarium oxysporum*, *Helminthosporium oryzae* and *Trichoderma viride*. In this investigation, the fungitoxicity of *C. limon* and *C. sinensis* EOs has been mainly tested on *Aspergillus* genera because they are frequent contaminants of medium and low moisture food. Metabolic activity of these microorganisms causes food spoilage and biosynthesize toxic secondary metabolites-mycotoxins: aflatoxins, ochratoxin A. *C. limon* and *C. sinensis* EOs has been also tested on *Fusarium* and *Penicillium* for their capability to synthesize mycotoxins as zearalenone, citrinin, ochratoxin A, fumonisin, patulin and deoxynivalenol. These toxigenic fungi should be studied in depth with regard to use *Citrus* EOs. Hence, *C. limon* and *C. sinensis* EOs would be necessitated for inhibition of the fungal contamination of foods.

Total phenolic content and antioxidant capacity

Data from the determination of total phenolic content of *C. limon* and *C. sinensis* EOs are summarized in Table 3. The total phenolic contents varied significantly ($p \leq 0.05$) between the two studied EOs, *C. limon* had higher total phenolic content (16.90 µg/mg) than *C. sinensis* (10.53 µg/mg). The antioxidant activity of *C. limon* and *C. sinensis* EOs were also tested. The concentrations that led to IC_{50} and the β-carotene oxidation inhibition *C. limon* and *C. sinensis* oils were 1570.10 and 752.26 µg/mg; 36.19 and 55.56 %, respectively ($p \leq 0.05$) (Table 3).

Previous studies showed a significant correlation between the antioxidant activity and total phenolic contents in herbs, vegetables and fruits [36-37]. In comparison with the obtained data, Prakash *at al*. [38] in his study found that the antioxidant activity of *Cananga odorata* EO was higher than *Commiphora myrrha* EO, although *C. myrrha* presented greater total phenolic content. Thus, antioxidant activity cannot be related to just phenolics.

Data of antioxidant activity exhibited a difference in DPPH radical scavenging between *C. limon* and *C. sinensis* EOs. This difference could be explained by the chemical composition of each EO. Additionally, free radical scavenging activity of EO may be attributed to the phenolic compounds presented in these EOs because it has

Table 3. Antioxidant activity and total phenolic of *C. sinensis* and *C. limon* EOs.

EOs	DPPH (IC_{50}) (µg/mL)	β-carotene/Linoleic acid inhibition (%)	Total phenolic content (µg/mg)
C. limon	1570.10±19.57[a]	36.19±0.64[a]	16.90±0.24[a]
C. sinensis	752.26±6.09[b]	55.56±0.64[b]	10.53±0.26[b]
BHT	306.15±4.49[c]	94.77±1.61[c]	nd

nd: not determined

Means in each column followed by different letters are significantly different ($p \leq 0.05$)

been reported that they have antioxidant activities against reactive oxygen species (ROS), or the interactions between the whole compounds [39-40]. On the other hand, the oxidation of β-carotene was considerably inhibited by *C. limon* and *C. sinensis* EOs. Jayashree and Subramanyam [41] in their investigation reported that oxidative stress causes AFB_1 production by *Aspergillus* spp. In later works, other authors as Narasaiah *et al*.[42]; Zjalic *et al*.[43] and Kim *et al*.[44], proved that biosynthesis of aflatoxins is really related to oxidative stress and peroxidation. Ferreira *et al*.[2] reported that the efficacy of *Curcuma longa* L. and curcumin on aflatoxins production may be attributed to the inhibition ternary steps of aflatoxins synthesis, lipid peroxidation and oxygenation. Hua *et al*.[45] showed that phenolics reveal inhibitory activity on AFB_1 production by *A. flavus*. Thereby, the efficacy of *C. limon* and *C. sinensis* EOs on AFB_1 production may be related to their antioxidant nature.

Phytotoxicity assay

During this investigation, the effects of *C. limon* and *C. sinensis* EOs on the germination and seedling growth of AS 81189 A (Ain Abid) and HD 1220 (Hiddab) were evaluated. As shown in Table 4, *C. limon* and *C. sinensis* EOs diminished significantly the germination of AS 81189 A (Ain Abid) seeds compared to the control (< 0.05), while, no significant decrease for HD1220 (Hiddab) seeds compared to the control (> 0.05). The length of radicles of AS 81189 A (Ain Abid) was significantly diminished in the seeds treated with *C. limon* and *C. sinensis* EOs ($p < 0.05$), but the effect of EOs on the length of plumules of AS 81189 A (Ain Abid), radicles and plumules of HD1220 (Hiddab) was non significative compared to the control (> 0.05) .

C. limon and *C. sinensis* EOs did not exhibit a potent phytotoxicity. Thus, the EOs may be only recommended for food commodities stored for

Table 4. Influence of *C. limon* and *C. sinensis* EOs on seed germination and seedling growth

Samples	Germination (%)	Seedling growth (mm) Radicle	Plumule
HD1220 (Hiddab)			
C. limon EO	80.00±4.00	52.66±3.92	43.70±1.70
C. sinensis EO	77.33±3.05	45.50±1.81	38.50±2.50
Control	92.66±3.05	67.70±2.98	57.83±1.30
AS 81189 A (Ain Abid)			
C. limon EO	72.66±3.0	60.06±4.24	52.96±6.16
C. sinensis EO	571.33±3.05	59.73±2.07	49.03±2.25
Control	90.66±4.16	69.40±2.98	60.30±4.59

consumption purpose.

Many authors studied the phytotoxicity of some EOs. Five plants (*Origanum majorana* L., *Coriandrum sativum* L., *Hedychium spicatum*, *Commiphora myrrha*, and *Cananga odorata*) were tested for their phytotoxicity on chickpea. They were found to be non phytotoxic [38]. Shukla *et al.*[46] tested the effect of *Callistemon lanceolatus* EO and its major component 1, 8-cineole on chickpea seed germination.

The authors noted that there was no adverse effect, suggesting their non-phytotoxic nature. Similar type of results were obtained by Kedia *et al.*[47], who reported that cumin EO revealed no phytotoxic effect on germination of wheat and chickpea seed. Until now, there are no published data on the effect of *Citrus* EOs and particularly *Citrus limon* var. Eureka and *Citrus sinensis* var. Valencia EOs on the germination and seedling growth of wheat.

Conclusion

The Algerian *C. limon* and *C. sinensis* EOs can inhibit fungal growth and AFB_1 production. They also showed fungitoxic spectrum against some isolated fungi, antioxidant activity and phytotoxicity. These findings should be taken into consideration by industries to exploit *Citrus* EOs as natural food additives for food items.

Acknowledgements

We are thankful to Mr Tarek Benabdelkhader and Laboratoire de Biotechnologies Végétales appliquées aux plantes aromatiques et médicinales (BVpam) de la Faculté des Sciences et Techniques de Saint Etienne, France for Gas chromatography-mass spectrometry (GC-MS) analsis and to Omrane Toumatia for the statistical analysis. Thanks to the Grupo de Investigación A06_17R (Gobierno de Aragón-FEDER, Spain) for financial support.

References

1. **Nguefack, J., Dongmo, J.B.L., Dakole, C.D., Leth, V., Vismer, H.F., Torp, J. et al. (2009).** Food preservative potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against mycotoxigenic fungi. Int. J. Food Microbiol. 131: 151-156.
2. **Ferreira, F.D., Kemmelmeier, C., Arrotéia, C.C., da Costa, L.C., Mallmann, C.A., Janeiro, V., Ferreira, F.M.D., Mossini, S.A.G., Silva, E.L. and Machinski, M. (2013).** Inhibitory effect of the essential oil of *Curcuma longa* L. and curcumin on aflatoxin production by *Aspergillus flavus* Link. Food Chem. 136: 789-793.
3. **Shukla, R., Kumar, A., Singh, P. and Dubey, N.K. (2009).** Efficacy of *Lippia alba* (Mill.) N.E. Brown essential oil and its monoterpene aldehyde constituents against fungi isolated from some edible legume seeds and aflatoxin B1 production. Int. J. Food Microbiol. 135: 165-170.
4. **Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Bagher Rezaee, M., Jaimand, K., Alinezhad, S., Saberi, R. and Yoshinari, T. (2009).** Chemical composition and antiaflatoxigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils. Food control. 1018-1024.
5. **Tzortzakis, N.G. (2009).** Impact of cinnamon oil-enrichment on microbial spoilage of fresh produce. Innov. Food Sci and Emerg. Technol. 10 : 97-102
6. **Burt, S. (2004).** Essential oils: their antibacterial properties and potential applications in foods: a review. Int. J. Food Microbiol. 94: 223-253.
7. **Adams, R.P. (2007).** Identification of essential oil components by gas chromatography/ mass spectrometry. Carol Stream, IL, USA: Allured Publishing Corporation.
8. **Pitt, J.I. and Hocking, A.D. (1997).** Fungi and food spoilage. Blackie Academic and Professional, London.
9. **Mishra, P.K., Singh, P., Prakash, B., Kedia, A. and Dubey, N.K. (2013).** Assessing essential oil components as plant-based preservatives against fungi that deteriorate herbal raw materials. Int. Biodeter. Biodegr. 80: 16-21.

10. **Singh, P., Srivastava, B., Kumar, A., Kumar, R., Dubey, N.K. and Gupta, R. (2008).** Assessment of *Pelargonium graveolens* oil as plant-based antimicrobial and aflatoxin suppressor in food preservation. J .Sci. Food Agric. 88: 2421-2425.
11. **José Velázquez-Nuñez, M., Avila-Sosa, R., Palou, E. and López-Malo, A. (2013).** Antifungal activity of orange (*Citrus sinensis* var. Valencia) peel essential oil applied by direct addition or vapor contact. Food Control. 31: 1-4.
12. **Shukla, R., Kumar, A., Prasad, C.S., Srivastava, B. and Dubey, N.K. (2008).** Antimycotic and antiaflatoxigenic potency of *Adenocalymma alliaceum* Miers. on fungi causing biodeterioration of food commodities and raw herbal drugs. Int. Biodeter. Biodegr. 62: 348-351.
13. **Tian, J., Huang, B., Luo, X., Zeng, H., Ban, X., J, He. and Wang Y. (2012).** The control of *Aspergillus flavus* with *Cinnamomum jensenianum* Hand.-Mazz essential oil and its potential use as a food preservative. Food Chem, 130: 520-527.
14. **Dewanto, V., Wu, X., Adom, K.K. and Liu, R.H. (2002).** Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agr. Food Chem. 50: 3010-3014.
15. **Brand-Williams, W., Cuvelier, M. E. et al. (1995).** Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss. Technol, 28: 25-30.
16. **Miraliakbari, H. and Shahidi, F . (2008).** Antioxidant activity of minor components of tree nut oils. Food Chem. 111 (2): 421-427.
17. **Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M. and Mete, E. (2008).** Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and *p*-cymene. Bioresource Technol. 99: 8788-8795.
18. **Djenane, D. (2015)**. Cheminal profile, Antimicrobial and Antioxidant Activity of Algerian Citrus Essential Oils and Their Application in *Sardina pilchardus*. Foods. 4: 208-228.
19. **Tu, M.N.T., Onishi, Y., Choi, H.S., Kondo, Y., Bassore, S.M., Ukeda, H. and Sawamura, M. (2002).** Characteristic odor components of *Citrus sphaerocarpa* Tanaka (Kabosu) cold-pressed peel oil. J. Agr. Food Chem. 50: 2908-2913.
20. **Ahmad, M.M., Rehman, S., Iqbal, Z., Anjum, F.M. and Sultan, J.I. (2006).** Genetic variability to essential oil composition in four citrus fruit species. Pak. J. Bot. 38: 319-324.
21. **Sharma, N. and Tripathi, A. (2008).** Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. Microbiol. Res. 163: 337-344.
22. **Singh, P., Shukla, R ., Prakash, B ., Kumar, A., Singh, S., Mishra, K.P. and Dubey, N.K. (2010).** Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of *Citrus maxima* Burm. and *Citrus sinensis* (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. Food Chem. Toxicol. 48: 1734-1740.
23. **Filipowicz, N., Kaminski, M., Kurlenda, J., Asztemborska, M. and Ochocka, J.R. (2003).** Antibacterial and antifungal activity of juniper berry oil and its selected components. Phytother. Res. 17: 227-231.
24. **Terzi, V., Morcia, C., Faccioli, P., Vale, G., Tacconi, G. and Malnati, M. (2007).** *In vitro* antifungal activity of the tea tree (*Melaleuca alternifolia*) essential oil and its major components against plant pathogens. Lett. Appl. Microbiol. 44: 613-618.
25. **Souza, E.L.D., Lima E.D.O., Freire, K.R.D.L. and Sousa, C.P.D. (2005).** Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. Braz. Arch .Biol .Technol. 48: 245-250.
26. **Hammer, K., Carson C. and Riley, T. (2003).** Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J. Appl. Microbiol. 95(4): 853-860.
27. **Cox, D., Mann, M., Markham, L., Bell, C., Gustafson, E., Warmington, R. and Wyllie, G.**

(2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J. App. Microbiol. 88: 170-175.

28. **Deba, F., Xuan, T., Yasuda, M. and Tawata, S. (2008).** Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa Linn*. var. Radiata. Food Contr. 19: 346-352.
29. **Chao, L.K., Hua, K.F., Hsu, H.Y., Cheng, S.S., Lin, J.Y. and Chang, S.T. (2005).** Study on the anti-inflammatory activity of essential oil from leaves of *Cinnamomum osmophloeum*. J. Agr. Food Chem. 53: 7274-7278.
30. **Kumar, A., Shukla, R., Singh, P., Prasad, C.S. and Dubey, N.K. (2008).** Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. Innov. Food. Sci. Emerg. Technol. 9: 575-580.
31. **Kalemba, D. and Kunicka, A. (2003).** Antibacterial and antifungal properties of essential oils. Curr. Med. Chem. 10: 813-829.
32. **Reddy, K.R.N., Reddy, C.S. and Muralidharan, K. (2009).** Potential of botanicals and bio-control agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. Food Control. 20: 173-178.
33. **Mishra, P.K., Shukla, R., Singh, P., Prakash, B. and Dubey, N.K. (2012).** Antifungal and antiaflatoxigenic efficacy of *Caesulia axillaris* Roxb. Essential oil against fungi deteriorating some herbal raw materials, and its antioxidant activity. Ind . Crops Prod. 36: 74- 80.
34. **Vilela, G.R., de Almeida, G.S ., D'Arce, M.A.B.R., Moraes, M.H.D., Brito, J.O., da Silva, M.F.d.G.F., Silva, S.C., de Stefano Piedade, S.M., Calori-Domingues, M.A. and da Gloria, E.M. (2009).** Activity of essential oil and its major compound, 1,8-cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. J. Stored Prod. Res. 45: 108-111.
35. **Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J. and Pérez-Álvarez, J. (2008).** Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Food Control. 19: 1130-1138.
36. **Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. (1998).** Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agr. Food Chem. 46: 4113-4117.
37. **Dorman, H.J.D., Bachmayer, O., Kosar, M. and Hiltunen R. (2004).** Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. J. Agr. Food Chem. 52: 762-770.
38. **Prakash, B., Singh P., Kedia, A. and Dubey, N.K. (2012).** Assessment of some essential oils as ood preservatives based on antifungal, antiaflatoxin, antioxidant activities and *in vivo* efficacy in food system. Food Res. Int. 49: 201-208.
39. **Lu, Y. and Foo, L.Y. (2000).** Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chem. 68: 81-85.
40. **Sreenivasulu, N., Grimm, B., Wobus, U. and Weschke, W. (2000).** Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). Plant Physiol. 109:435-442.
41. **Jayashree, T. and Subramanyam, C. (2000).** Oxidative stress as a prerequisite for aflatoxin production by *Aspergillus parasiticus*. Free Radical. Bio. Med. 29: 981-985.
42. **Narasaiah, K.V., Sashidhar, R. and Subramanyam, C. (2006).** Biochemical analysis of oxidative stress in the production of aflatoxin and its precursor intermediates. Mycopathologia. 162: 179-189.
43. **Zjalic, S., Reverberi, M., Ricelli, A., Granito, V.M., Fanelli, C. and Fabbri, A.A. (2006).** Trametes versicolor: A possible tool for aflatoxins control. Int. J. Food Microbiol. 107: 243-246.
44. **Kim, J.H., Yu, J., Mahoney, N., Chan, K.L., Molyneux, R.J., Varga, J. et al. (2008).**

Elucidation of the functional genomics of antioxidant-based inhibition of aflatoxin biosynthesis. Int. J. Food Microbiol. 122: 49-60.
45. **Hua, S.S.T., Grosjean, O.K. and Baker J.L. (1999).** Inhibition of aflatoxin biosynthesis by phenolic compounds. Lett. Appl. Microbiol. 29: 289-291.
46. **Shukla, R., Singh, P., Prakash, B. and Dubey, N.K. (2012).** Antifungal, aflatoxin inhibition and antioxidant activity of *Callistemon lanceolatus* (Sm.) Sweet essential oil and its major component 1,8-cineole against fungal isolates from chickpea seeds. Food Control. 25: 27-33.
47. **Kedia, A., Prakash, B., Mishra, P.K. and Dubey, N.K. (2014).** Antifungal and antiaflatoxigenic properties of *Cuminum cyminum* (L.) seed essential oil and its efficacy as a preservative in stored commodities. Int. J. Food Microbiol. 168-169, 1-7.
48. **Saroglou, V., Arfan, M., Shabir, A., Hadjipavlou-Litina, D. and Skaltsa H. (2007).** Composition and antioxidant activity of the essential oil of Teucriumroyleanum Wall. Ex Benth growing in Pakistan. Flavour Frag. J. 22 (2): 154-157.
49. **Wu, P., Kuo, M. C., Hartman, T.G., Rosen, R.T. and Ho, C.T. (1991).** Free and glycosidically bound aroma compounds in pineapple (*Ananascomosus* L. Merr.). J. Agr. Food Chem. 39(1): 170-172.
50. **Vérité, P., Nacer, A., Kabouche, Z. and Seguin E. (2004).** Composition of seeds and stems essential oils of *Pituranthosscoparius* (Coss. Dur.) Schinz Flavour. Frag. J. 19: 562-564.
51. **Verzera, A., Trozzi, A., Zappala, M., Condurso, C. and Cotroneo A. (2005).** Essential Oil Composition of *Citrus meyerii* Y. Tan. and *Citrus medica* L. cv. Diamante and Their Lemon Hybrids. J. Agr. Food Chem. 53(12): 4890-4894.
52. **Saroglou, V., Dorizas, N., Kypriotakis, Z. and Skaltsa, H.D. (2006).** Analysis of the essential oil composition of eight Anthemis species from Greece. J. Chromatogr. A. 1104(1-2): 313-322.
53. **Fang, Y. and Qian, M. (2005).** Aroma compounds in Oregon Pinot Noir wine determined by aroma extract dilution analysis (AEDA). Flavour Frag. J. 20 (1): 22-29.
54. **BinJantan, I., Yassin, M.S.M., Chin, C.B., Chen, L.L. and Sim, N.L. (2003).** Antifungal activity of the essential oils of nine *Zingiberaceae* species. Pharm. Biol. 41(5): 392-397.
55. **Chosson, E., Vérité, P., Blanckaert, A., Seguin, E., Litaudon, M. and Sévenet, T. (2003).** Non polar compounds from the bark of *Sarcomelicope follicularis*. Biochem. Syst. Ecol. 31(10): 1185-1188.