Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Innovative Food Science and Emerging Technologies 11 (2010) 410–414

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Essential oils composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumonisin production by *Fusarium verticillioides* ☆

José S. Dambolena [a,*], Maria P. Zunino [a], Abel G. López [b], Héctor R. Rubinstein [c], Julio A. Zygadlo [a,1], Julius W. Mwangi [d], Grace N. Thoithi [d], Isaac O. Kibwage [d], Josphat M. Mwalukumbi [d], Samuel T. Kariuki [e]

[a] *Instituto Multidisciplinario de Biología Vegetal (IMBiV-CONICET), Cátedra de Química Orgánica, FCEFyN-UNC, Avenida Vélez Sarsfield 1611, X5016GCA, Cordoba, Argentina*
[b] *Instituto de Ciencia y Tecnología de los Alimentos (ICTA), FCEFyN-UNC, Avenida Vélez Sarsfield 1611, X5016GCA, Cordoba, Argentina*
[c] *CIBICI (CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina*
[d] *School of Pharmacy, University of Nairobi, P.O. Box 19676-00202, Nairobi, Kenya*
[e] *Department of Biological Sciences, Egerton University, P. O. Box 536, Njoro, Kenya*

A R T I C L E I N F O

Article history:
Received 14 May 2009
Accepted 3 August 2009

Keywords:
Ocimum basilicum
Ocimum gratissimum
Lamiaceae
Basil
Composition
Eugenol
Antifungal
Antiradical
Antimycotoxicogenic activity

A B S T R A C T

This work investigated the constituents and the efficacy against *Fusarium verticillioides* infection and fumonisin production of essential oils of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from different locations in Kenya.

The oil of leaves and flowering tops of *O. basilicum* from Sagana contained mainly linalool (95%). The flowering tops and leaves from Yatta contained mainly camphor (32.6 and 31.0%, respectively) and linalool (28.2 and 29.3, respectively). Eugenol was the main constituent in the oil of *O. gratissimum* leaves from both Sagana (95.5%) and Yatta (70.1%). The oil of the flowering tops had significantly less eugenol. The main component of the oil of flowering tops from Yatta was Z-β-ocimene (34.1%). Oil from both species had some antifungal activity. The oils of *O. basilicum* and *O. gratissimum* from different locations showed chemical variation, antifungal activity, free radical scavenging capacity and antimycotoxicogenic property. These properties are attributed to the phenolic compound eugenol.

Industrial relevance: This manuscript gives the chemical composition and some biological effects of essential oil of two *Ocimum* species in Kenya namely *Ocimum basilicum* L. and *Ocimum gratissimum* L. The work reveals that there are chemovarieties of these plants in different locations in the country. Of significance is the presence of very high amounts of linalool in one chemovariety of *O. basilicum* while geranial and neral are major in another. The third variety contains almost equal amounts of camphor and linalool. The first two containing high quantities of linalool, geranial and neral could be cultivated after agronomic studies to provide essential oils useful in perfumery, soap or food industry while that containing camphor and linalool may find use in medicine.

Proper seed selection and good agricultural practice for *O. gratissimum* containing high amount of eugenol could serve as good substitute for cloves which grows best only in a few islands in East Africa. Clove oil due to its high eugenol content has many biological activities including those demonstrated in the present work, and many applications in medicine and commercial world.

There is need for more research on these plants especially on cultivation and commercial exploitation of the herbs.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Fungi of the genus *Fusarium* are widely found in plant debris and crop plants worldwide (Marasas, Kriek, Fincham, & Van Rensburg, 1984). Several species from this genus are economically relevant because, apart from their ability to infect and cause tissue destruction on important crops such as corn, wheat and other small grains on the field, they produce mycotoxins on the crops in the field and in storage grains. Fumonisins are mycotoxins produced mainly by the fungi *Fusarium verticillioides* and *Fusarium proliferatum*. Fumonisin B1 (FB1) is

☆ This paper was presented in part at The 12th Symposium of The Natural Product Research Network for Eastern and Central Africa (NAPRECA), Kampala, Uganda 22nd–25th July 2007.

* Corresponding author. Tel./fax: +54 351 4334439.
E-mail address: dambolena@mail.fcq.unc.edu.ar (J.S. Dambolena).
[1] Tel./fax: +54 351 4334439.

1466-8564/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ifset.2009.08.005

Author's personal copy

generally the most abundant member of the family of mycotoxins and is known to cause animal diseases including equine leucoencephalomalacia, porcine pulmonary edema and alter some diet and inmunobiological parameters in mice and rats (Theumer, Lopez, Masih, Chulze, & Rubinstein, 2002). Additionally, fumonisins are potent liver toxins in most animal species and are suspected human carcinogens (Nelson, Desjardins, & Plattner, 1993).

Aromatic plants are used in folk medicine as antimicrobial agent and their essential oils have been known to have antibacterial and antifungal proprieties (Pinto, Ribeiro Salgueiro, Cavaleiro, Palmeira, & Gonçalves, 2007). *Ocimum basilicum* L. and *Ocimum gratissimum* L. (*Ocimum suave* Willd., *Ocimum tomentosum* Oliv.) (Lamiaceae family) are grown in Africa. The last, is very common in disturbed areas such as secondary bushland, forest margins, grasslands and riverine sites. In dry areas it is always on higher hills (Agnew & Agnew, 1994). Both plants are used widely in East Africa, with *O. basilicum* root decoction being drunk by pregnant women for stomach pains and as an enema in constipation. Vapour from boiling leaves is inhaled for treatment of nasal and bronchial catarrh and the leaves are put in the house to drive away mosquitos (Kokwaro, 1980). In Asia, the leaves are considered stomachic, carminative and sudorific. Seeds have a slight laxative effect (Van Duong, 1993). *O. gratissimum* leaves are used for abdominal pains, sore eyes, ear troubles, coughs and blocked noses. An infusion of the leaves is used as a disinfectant and as an insecticide (Kokwaro, 1980). In Asia, it is used as a stomachic, diaphoretic and febrifuge (Van Duong, 1993).

Several strategies are used at controlling fungal growth and the mycotoxin synthesis in stored grains by chemical treatments with ammonia, acids and bases or with food preservatives by physical methods and by biological methods. These methods require sophisticated equipment and expensive chemicals or reagents. Therefore, it is important to find a practical, cost effective and non-toxic method to prevent fungal deterioration of stored grains (Reddy, Reddy, & Muralidharan, 2009). In recent years, particular interest has been focused on the potential application of plant essential oils (EOs) to prevent fungal growth and mycotoxins yield in the cereals and grain-based food. Some essential oils are classified as Food Additives by the FDA. A wide variety of EOs and many of their majority compounds, mainly mono and sesquiterpenes have been shown to have antifungal and antimycotoxins activities. Generally, the EOs possessing the strongest antifungal activities against food borne pathogens contain a high percentage of phenolic compounds such as carvacrol, eugenol, isoeugenol and thymol (Lambert, Skandamis, Coote, & Nychas, 2001).

In this study, we report for the first time, the chemical composition, antifungal, antiradical and antimycotoxigenic activities of the essential oils of *O. bassilicum* and *O. gratissimum* from different locations in Kenya.

2. Materials and methods

2.1. Plant material and oil isolation

Wild *O. basilicum* L. (Lamiaceae) was collected in July 2001 from Kariti, Kirinyaga District, Central Kenya and in February 2003 from both Sagana (Kirinyaga District) and Yatta (Machakos District, Eastern Kenya). *O. gratissimum* L. (Lamiaceae) was collected from both Sagana and Yatta in February 2003. Voucher specimens were identified by a taxonomist and deposited in the Herbarium at the School of Pharmacy, University of Nairobi. Semi-dried plant materials were hydro-distilled in Clevenger like apparatus, dried over anhydrous sodium sulphate and stored at 4 °C until analysis.

2.2. Gas chromatography analyses

Analyses were performed on a Shimadzu GC-R1A (FID) gas-chromatograph (GC), fitted with a fused silica capillary column coated with a stationary phase consisting of 5% phenyl 95% dimethylpolysiloxane (non-polar DB-5) (30 m × 0.25 mm i.d.; film coating thickness 0.25 μm). For the critical pair separation, a capillary column Supelcowax 10 (polar polyethylene glycol 100%) with the same GC operating conditions of DB-5 was used. The GC operating conditions were as follows: oven temperature was programmed from 40 °C to 230 °C at 2 °C/min, injector and detector temperatures were set at 240 °C. The carrier gas was nitrogen at a constant flow of 0.9 mL/min. Identification of the components was performed by comparison of their retention index (RI) with reference to a homologous series of *n*-alkanes (C_9–C_{25}) and with those of pure authentic samples. Gas chromatography-Mass spectrometry (GC-MS) analyses were performed with a Perkin Elmer Q-700 equipped with an apolar SE-30 capillary column, stationary phase 100% dimethylpolysiloxane, (30 m × 0.25 i.d. mm; film coating thickness 0.25 μm). The analytical conditions were: oven temperature from 40 °C to 230 °C at 2 °C/min; the carrier gas was helium at a constant flow of 0.9 ml/min and the ion source was maintained at 70 eV. The oil components were identified by two computer library MS searches (Wiley and Nist) using retention indices as a pre-selection routine, and visual inspection of the mass spectra from literature for confirmation (Adams, 1995).

2.3. Antifungal activity

For the evaluation of antifungal activities, experiments were performed according to a modified semisolid agar antifungal susceptibility method (SAAS) (Provine & Hadley, 2000). Five-milliliter sterile aliquots of semisolid brain–heart infusion broth (Difco Laboratories, Detroit, Michigan U.S.A.) containing 0.5% w/v agar (Bacto Agar, Difco Laboratories), pH 7.4 (without dextrose, buffer or indicator) were prepared, in 16 × 125 mm glass tubes. The oils were dissolved in dimethyl sulfoxide (DMSO), and then added to the different tubes in order to obtain oil concentrations of 0.1, 0.3, 0.5, 1, 2, 4, and 5 μl/ml in the culture medium. The final concentration of DMSO was adjusted to 4.5 μl/ml in each of the tube. Mixing of the volatile compounds with the medium was done at 45 °C, and then media were stored at 4 °C to solidify. As a control, an oil-free medium with a 4.5 μl/ml final concentration of DMSO was used. A conidia suspension (1 × 106 CFU/mL) prepared with a *F. verticillioides* (Sacc.) Nierenberg (ex *F. moniliforme* Sheldon) MRC 826 (Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC) , Tygerberg, South Africa) culture grown in V-8 juice agar for 2 weeks and Tween 20 at 2.5% (v/v) in sterile water, were used as inoculums. A standard loopful (0.001 ml) of this conidia suspension was inserted deeply into each tube by a centered down-up motion to form a two dimensional inoculum. Sterile mineral oil (0.5 ml) was layered on the inoculated medium to inhibit sporulation, and the tubes were then tightly capped. All cultures were incubated for 48 h at 28 °C or until good growth was apparent in the inoculated oil-free control. Within 48 h, when on visual inspection a good growth of the *F. verticillioides* in the oil-free medium was detected, the growth in all tubes was compared with that of the oil-free control. The growth was scored in the following manner: 4, growth comparable to that of the oil-free control; 3, growth approximately 75% that of the control; 2, growth approximately 50% that of the control; 1, growth 25% or less that of the control; and 0, no visible growth. For each treatment, 5 replicates were done.

2.4. Free radical scavenging activity

The antioxidant activity of the individual essential oil was tested by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Briefly, 350 μl of a 0.022% DPPH solution in methanol was added to a range of solutions at different concentrations (eleven serial, two-fold dilutions to give a final range of 10,000 to 9.8 μl/l) of each essential oil in methanol (1610 μl). Absorbance was determined at 517 nm, 30 min

Author's personal copy

after the addition of DPPH solution, and the percentage of activity calculated. Gallic acid was used as positive control (five two-fold serial dilutions with a final range of 0.1 to 0.0063 mM) (Gálvez, Martín-Cordero, Houghton, & Ayuso, 2005).

2.5. Effect of essential oils on fumonisin B1 production

The FB1 production was determined using healthy maize as substratum. Corn grain free from FB1 (300 g), was placed in 1.000-ml dark Erlenmeyer flasks at 35% humidity and sterilized for two consecutive days in autoclave for 15 min at 121 °C. Autoclaved maize was inoculated with 200 µl of conidia suspension of *F. verticillioides* prepared as described in testing for antifungal activity. Incubation lasted 28 days in the dark at 25 °C, with manual stirring the first 5 days. The EOs were applied on a sterilized paper disk Whatman No. 3 (14 mm diameter), which was placed over corn grain on the 5th day post-inoculation. The EOs concentrations used were 100 µl/kg of maize (100 ppm). Control flasks were prepared following the same procedure, however, no EOs were added on paper disk. Five replications of each treatment were done. The experiment was performed twice. Separation and purification of the toxin were performed in the fermented maize following the methodology of Voss, Plattner, Bacon and Norred (1990) modified.

2.6. Fumonisin B1 quantification

Briefly, after incubation, fermented maize was sterilized in an autoclave for 15 min at 121 °C and dried in a vacuum oven at 60 °C until constant weight was achieved. Later, 10 g of dried maize was finely ground. The FB1 was extracted with ultrapure water by shaking the powder and water for 2 h in an orbital shaker. The aqueous extracts were centrifuged at 9000*g*, and filtered through filter paper (Whatman no. 4, Whatman International, Maidstone, UK). Samples (100 ml) from the aqueous extracts were diluted with acetonitrile (100 µl). Before the quantification assays, the samples were diluted 1/50 with acetonitrile/water (1:1). The quantification of the diluted extracts was performed following the methodology proposed by Shephard, Sydenham, Thiel and Gelderblom (1990). Briefly, an aliquot (50 µl) of this solution was derivatized with 200 µl of *o*-phthaldialdehyde. This solution was obtained by adding 5 ml of 0.1 M sodium tetraborate and 50 µl of 2-mercaptoethanol to 1 ml of methanol containing 40 mg of *o*-phthaldialdehyde. The derivatized samples were analyzed by means of a Hewlett Packard HPLC equipped with a fluorescence detector. The wavelengths used were 335 nm and 440 nm for excitation and emission, respectively. An analytical reversal phase column C18 (150 mm × 4.6 mm internal diameter and 5 µm particle size) connected to a precolumn C18 (20 mm × 4.6 mm and 5 µm particle size) was also used. The mobile phase was methanol, NaH2PO4 0.1 M (75:25); the pH was set at 3.35 ± 0.2 with orthophosphoric acid, and a flow rate of 1.5 ml/min. was used. The quantification of fumonisin B1 was carried out by comparing the peak areas obtained from watered extracts with those corresponding to the standards of 10.5; 5.25 and 2.625 µg/ml FB1 (PROMEC, Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg; Republic of South Africa).

2.7. Statistical evaluation

Data from these studies were analyzed by one-way analysis of variance (ANOVA) and Fisher Multiple Comparison test. Results giving *P* values < 0.05 were considered significantly different.

3. Results and discussion

Semi-dried *O. basilicum* yielded 0.4% (Yatta leaves), 1.3% (Yatta flowering tops), 1.1% (Sagana flowering tops), 1.9% (Sagana leaves) and 0.2% (Kariti leaves) oil. *O. gratissimum* yielded 2.9 % (Yatta leaves), 0.9% (Yatta flowering tops), 0.2% (Sagana leaves) and 1.4% (Sagana flowering tops).

The oil of *O. basilicum* from Sagana contained mainly the monoterperne linalool (more than 95% in both leaves and flowering tops), while that from the leaves of *O. basilicum* from Kariti contained geranial (49.6%) and neral (30.9%) as the main constituents (Table 1). The flowering tops and leaves from Yatta contained mainly camphor and linalool (about 30% of each). The oils studied did not contain some of the compounds that have been reported in *O. basilicum* from other countries to be in high amounts, such as methyl cinnamate, eugenol methyl ester (Brophy & Jogia, 1986; Thoppil, Tajo, & Minija, 1998), chavicol methyl ester (Pino, Roncal, Rosado, & Goiie, 1994), eugenol and estragol (Fandohan et al., 2004). Variation in constituents of *O. basilicum* has previously been reported by Kartnig and Simon (1986), who found that linalool, methyl chavicol and eugenol varied widely with harvest dates and varieties.

Brophy and Jogia (1986) reported on two chemotypes of *O. basilicum* from Fiji, one containing mainly methyl cinnamate, while the other contained mainly methyl eugenol and linalool. Our study shows that the oils from the three different locations in Kenya are indeed chemically variable, and the amount of linalool found in *O. basilicum* from Sagana is much higher than that previously reported in oils from Turkey (43.7%), India (41.6%) Benin (33%) and Fiji (22.0 %) (Brophy & Jogia, 1986; Fandohan et al., 2004; Thoppil et al., 1998). It has been observed that polymorphism in the species of *basilicum* is

Table 1
Chemical composition of the essential oils of *Ocimum basilicum* from Yatta, Sagana and Kariti.

R.I.[a]	Component[b]	Yatta (L)	Yatta (FT)	Sagana (L)	Sagana (FT)	Kariti (L)	Methods of identification
930	α-thujene	0.1	t	t	t	–	MS_1
938	α-pinene	–	t	t	t	–	MS_2 Co
955	Camphene	–	–	t	t	–	MS_1
975	Sabinene	2.2	1.0	t	t	–	MS_1
979	β-pinene	–	t	–	–	–	MS_2 Co
990	Myrcene	–	t	t	t	–	MS_2 Co
1004	α-phellandrene	0.2	2.2	t	t	–	MS_2 Co
1018	α-terpinene	0.9	t	–	–	–	MS_1
1025	p-cymene	0.3	1.3		t	0.3	MS_2 Co
1030	limonene[c]	2.7	4.7	t	t	0.2	MS_2 Co
1030	β-phellandrene[c]	–	–	–	–	0.4	MS_2 Co
1033	1,8-cineole[c]	0.4	t	t	t	–	MS_2 Co
1037	Z β ocimene	–	–	t	t	–	MS_2 Co
1061	γ-terpinene	1.5	0.5	–	–	–	MS_1
1085	Fenchone	–	–	–	–	0.2	MS_1
1099	Linalool	28.2	29.3	95.7	98.9	0.2	MS_2 Co
1146	Camphor	32.6	31.0	–	–	–	MS_2 Co
1155	Citronellal	–	–	–	–	1.6	MS_1
1170	Borneol	1.9	1.9	–	–	–	MS_2 Co
1177	Terpinen-4-ol	12.0	9.0	–	–	–	MS_1
1196	Myrtenal[c]	0.4	t	–	–	–	MS_1
1196	Myrtenol[c]	0.7	t	–	–	–	MS_1
1226	Citronellol	–	–	–	–	6.5	MS_1
1239	Neral	–	–	–	–	30.9	MS_2 Co
1252	Geraniol	–	–	–	–	3.7	MS_2 Co
1265	Geranial	–	–	–	–	49.6	MS_2 Co
1350	α-cubenene	t	0.3	–	–	–	MS_1
1391	β-elemene	t	t	–	t	–	MS_1
1419	E-caryophyllene	5.1	7.1	t	t	1.0	MS_1
1441	Aromadendrene	4.3	2.0	–	–	–	MS_1
1455	α-humulene	0.3	4.3	–	–	–	MS_1
1460	Aromadendrene (allo)	2.7	1.8	–	–	–	MS_1
1640	Cadinol tau	t	t	–	–	–	MS_1
	Total	99.7	91.7	95.7	98.7	94.6	

Components are listed in order of elution on DB-5 column. a: retention indices (RI) relative to homologous alkanes. b: Percentages were calculated from the peak area without correction. c: Identification based on RI and mass spectrometry on a Supelcowax 10 capillary column. MS_1: identical with published data. MS_2: identical with those of pure reference compounds. Co: co-injection with authentic compounds. t: trace < 0.1%. FT: Flowering tops. L: Leaves.

Author's personal copy

responsible for the very great number of sub-species, varieties and forms (Guenther, 1952).

Eugenol was the main constituent in the essential oil of *O. gratissimum* leaves from both Sagana (95.5%) and Yatta (70.1%) and in the flowering tops of Sagana (64.0%) (Table 2). This is largely in agreement with previous work which has shown eugenol as the major constituent of the essential oil of *O. gratissimum* (Dube, Upadhyay, & Tripathi, 1989; Yu & Cheng, 1986). However, the oil of flowering tops from Yatta differed significantly as it contained Z-β ocimene as the main component (34.1%) and only 33.2 % of eugenol. Besides, Fandohan et al. (2004) reported that essential oil from *O. gratissimum* of Benin contained *p*-cymene (22%), its precursor—terpinene (15%), and thymol (17%) as the main components.

All the EOs showed some inhibitory effects on growth of *F. verticillioides* (Table 3). However, the extent of inhibition was widely dependent upon the composition and the concentration of EOs. The oil of *O. gratissimum* that had high content of eugenol (Sagana and Yatta leaves) showed significant activity (MIC 0.3 µl/ml) against *F. verticillioides* (Table 3). The low activity of the oil of flowering tops of *O. gratissimum* from Yatta can be attributed to their low eugenol content (33.2%). The oil of *O. basilicum* had moderate antifungal activity ranging between 1 and 2 µl/ml. The inhibitory effect of the oils increased as oil concentration and eugenol content increased. The antifungal activities of these oils might be attributable to the main compounds that they contain. Several authors have demonstrated high antifungal activity of eugenol in different fungi specie (Cheng, Liu, Chang, & Chang, 2008; Serrano, Martínez-Romero, Castillo, Guillén, & Valero, 2005).

Table 2
Chemical composition of the essential oils of *Ocimum gratissimum* from Yatta and Sagana.

R.I.[a]	Component[b]	Yatta (L)	Yatta (FT)	Sagana (L)	Sagana (FT)	Methods of identification
938	α-pinene	0.2	t	t	0.2	MS_2 Co
955	Camphene	t	t	t	t	MS_1
975	Sabinene	t	t	t	t	MS_1
980	1-octen-3-ol	0.3	0.2	–	–	MS_2 Co
990	Myrcene	t	t	t	t	MS_2 Co
1018	α-terpinene	–	t	–	–	MS_1
1025	p-cymene	t	–	t	0.8	MS_2 Co
1030	Limonene	0.2	0.7	t	0.3	MS_2 Co
1033	1,8-cineole	0.1	t	t	0.6	MS_2 Co
1037	Z-β ocimene	0.2	34.1	t	2.7	MS_2 Co
1050	E-β ocimene	0.2	–	t	0.7	MS_2 Co
1061	γ-terpinene	t	–	–	–	MS_1
1090	Terpinolene	–	0.1	–	–	MS_1
1099	Linalool	–	7.5	–	0.9	MS_2 Co
1146	Camphor	1.9	8.5	–	–	MS_2 Co
1162	E-β terpineol	2.7	–	–	T	MS_1
1177	Terpinen-4-ol	–	–	–	0.6	MS_1
1239	Neral	–	2.8	–	–	MS_2 Co
1350	α-cubenene	–	–	–	0.2	MS_1
1360	Eugenol	70.1	33.2	95.5	64.0	MS_2 Co
1387	β-bourbonene[c]	–	–	t	0.6	MS_1
1387	β-cubenene[c]	–	0.4	t	0.4	MS_1
1391	β-elemene	0.7	–	t	t	MS_1
1419	E-caryophyllene	0.3	–	t	2.2	MS_1
1433	β-gurjunene	0.2	–	t	–	MS_1
1436	Trans-α-bergamotene	0.7	–	t	–	MS_1
1456	α-humulene	11.9	6.0	t	1.0	MS_1
1499	Viridiflorene	t	–	–	2.5	MS_1
1506	β-bisabolene	–	–	t	10.2	MS_1
1515	Z-γ bisabolene	–	–	–	6.9	MS_1
1523	δ-cadinene	t	–	–	0.4	MS_1
1746	E-Z farnesol	5.5	–	–	–	MS_1
	Total	94.9	95.4	95.5	95.9	

Components are listed in order of elution on DB-5 column. a: retention indices (RI) relative to homologous alkanes. b: Percentages were calculated from the peak area without correction. c: Identification based on RI and mass spectrometry on a Supelcowax 10 capillary column. MS_1: identical with published data. MS_2: identical with those of pure reference compounds. Co: co-injection with authentic compounds. t: trace <0.1%. FT: Flowering tops. L: Leaves.

Table 3
Antifungal activity of *Ocimum basilicum* and *Ocimum gratissimum* oils against *Fusarium verticillioides*.

Essential oil	Concentrations (µl/ml of culture medium)						
	5	4	2	1	0.5	0.3	0.1
Ocimum basilicum Yatta FT	0	1	1	1[a]	2	4	4
Ocimum basilicum Yatta L	0	1	1	1[a]	2	4	4
Ocimum basilicum Sagana L	0	1	1	1[a]	2	3	4
Ocimum basilicum Sagana FT	0	1	1[a]	2	2	3	4
Ocimum gratissimum Yatta L	1	1	1	1	1	1[a]	4
Ocimum gratissimum Yatta F	1[a]	2	3	4	4	4	4
Ocimum gratissimum Sagana L	1	1	1	1	1	1[a]	3

0: No visible growth. 1: Growth 25% or less than control. 2: Growth approximately 50% of the control. 3: Growth approximately 75% of the control, 4: Growth about 100% of control. FT: Flowering tops. L: Leaves.
[a] Score of minimal inhibitory concentration. $n = 5$.

The antifungal activity is consistent with other work which has shown that *O. basilicum* oil has antifungal and antibacterial activity (Hussain, Anwar, Hussain Sherazi, & Przybylsk, 2008; Thoppil et al., 1998). Among the components of essential oils, the oxygenated-containing compounds have shown a stronger antifungal activity (Dube et al., 1989) compared to those of hydrocarbonated ones. These results are in agreement with Kurita and Koike (1983), who reported antifungal activity of essential oils according to their major component following the rule: phenols > alcohols > aldehydes > ketones > esters > hydrocarbons. Therefore, our results corroborate the strong antifungal activity of phenolic components rich-EOs.

The mechanism of phenolic compounds toxicity such as thymol, carvacrol and eugenol includes enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Cowan, 1999). Their possible consequence is damage to membrane integrity, which could affect pH homeostasis and equilibrium of inorganic ions (Lambert et al., 2001).

The effects of EOs on FB1 production by *F. verticillioides* were studied (Fig. 1). When maize was treated with *O. basilicum* oils no

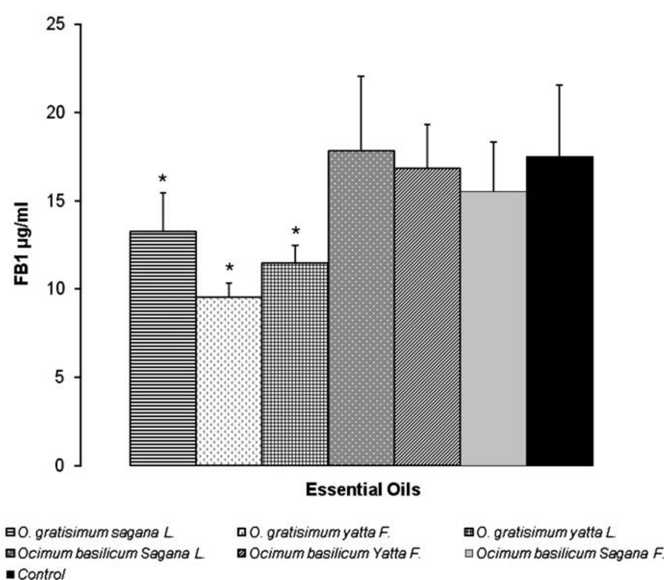

Fig. 1. Effects of essential oils on fumonisin FB_1 production by *Fusarium verticillioides*. The essential oils were evaluated at final concentrations of 100 ppm. Five replications were done for each treatment. $^*P < 0.05$, With respect to the control.

Author's personal copy

Table 4
Activity of the different essential oils against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical.

Essential oils	IC_{50} (µl/l)
Ocimum basilicum L. from Yatta (leaves)	1150.9
Ocimum basilicum L. from Sagana (leaves)	>10,000
Ocimum basilicum L. from Sagana (flowering tops)	>10,000
Ocimum gratissimum L. from Yatta (leaves)	9.8
Ocimum gratissimum L. from Yatta (flowering tops)	15.4
Ocimum gratissimum L. from Sagana (leaves)	6.8
Linalool	>10,000
Eugenol	2.3

IC_{50}: Concentration of the solution required to give a 50% decrease in absorbance compared to that of a blank solution.

effects were observed in the FB1 biosynthesis. *O. gratissimum* essential oils were found to induce a significant inhibitory effect on FB1 production with respect to control ($P<0.05$). The high activity of the oil of *O. gratissimum* can be attributed to their high eugenol content (Table 2). Several authors have reported high inhibitory activity of phenolic components. This is in agreement with our finding in that rich eugenol EOs had significant inhibitory effect on FB1 biosynthesis by *F. verticillioides*. Besides, our results showed a direct relationship between inhibitory effects of essential oils on fungal growth and FB1 production. This is largely in agreement with previous work which reports the same relationship (Dambolena et al., 2008).

Fandohan et al. (2004) showed that *O. basilicum* essential oil of Benin possess significant inhibitory effect on FB1 production. However, this essential oil contains 22% of eugenol while the essential oil of *O. basilicum* here studied, has nothing.

The oil of *O. gratissimum* that had high content of eugenol (Sagana and Yatta leaves) showed significant antiradical effect in DPPH assay (Table 4). The high activity of the oil of *O. gratissimum* can be attributed to their high eugenol content (Table 2). This was corroborated by the pure compounds tested, eugenol, which displayed potent free radical scavenging capacity ($IC_{50}=2.3$ µl/l) (Table 4). The key role of phenolic compounds as scavengers of free radical is emphasised in several reports. On the other hand, the essential oil of *O. basilicum* which is rich in linalool, have not antiradical activity. Linalool, which lacks a phenol ring, does not have antiradical activity because its IC_{50} was >10 µl/ml (Table 4).

Many authors have reported that antioxidant compounds are used for controlling fungal growth and mycotoxin biosynthesis (Torres, Ramirez, Arroy, Chulze, & Magan, 2003). Jayashree and Subramanyam (1999) reported that the antiaflatoxigenic activity of eugenol is due to the inhibition of lipid peroxidation. The oil of *O. gratissimum* that had high content of eugenol, an antioxidant compounds, have showed a strong inhibition of fumonisin production (Fig. 1). Thus, this antitoxigenic activity might be attributed to their antioxidant properties.

The oils of *O. basilicum* and *O. gratissimum* from different locations in Kenya showed chemical variation and some antifungal activity. *Ocimum gratissimum* oil exhibited strong free radical scavenging and antimycotoxicogenic capacity, which can be attributed to its high eugenol content.

This study has demonstrated that *Ocimum* essential oils from Kenya can also serve as alternative means to prevent or control fungal attack and the presence of mycotoxin in stored corn and upon foodstuff.

Acknowledgements

JSD is a fellow of CONICET. MPZ and JAZ are researchers of CONICET. The authors are indebted to CONICET and SECyT-UNC for partial financial support.

References

Adams, R. (1995). *Identification of essential oil components by gas chromatography and mass spectroscopy*. Carol Stream, Illinois: Allured Publ. Corp.
Agnew, A., & Agnew, S. (1994). *Upland Kenya wild flowers* (pp. 296). Nairobi, Kenia: East Africa History Society.
Brophy, J., & Jogia, M. (1986). Essential oils from Fijian *Ocimum basilicum* L. *Flavour and Fragrance Journal*, *1*, 53−55.
Cheng, S. S., Liu, J. Y., Chang, E. H., & Chang, S. T. (2008). Antifungal activity of cinnamaldehyde and eugenol congeners against wood-rot fungi. *Bioresource Technology*, *99*, 5145−5149.
Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*, 564−568.
Dambolena, J. S., López, A. G., Cánepa, M. C., Theumer, M. G., Zygadlo, J. A., & Rubinstein, H. R. (2008). Inhibitory effect of cyclic terpenes (limonene, menthol, menthone and thymol) on *Fusarium verticillioides* MRC 826 growth and fumonisin B1 biosynthesis. *Toxicon*, *51*, 37−44.
Dube, S., Upadhyay, P., & Tripathi, S. (1989). Antifungal, physicochemical and insect-repelling activity of the essential oil of *O. basilicum*. *Canadian Journal of Botany*, *67*, 2085−2087.
Fandohan, P., Gbenou, J. D., Gnonlonfin, B., Hell, K., Marasas, W. F. O., & Wingfield, M. J. (2004). Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn. *Journal of Agricultural Food Chemistry*, *52*, 6824−6829.
Gálvez, M., Martín-Cordero, C., Houghton, P., & Ayuso, M. (2005). Antioxidant activity of methanol extracts obtained from *Plantago* species. *Journal of Agricultural Food Chemistry*, *53*, 1927−1933.
Guenther, E. (1952). *The essential oils. Vol III* (pp. 399−413). London: D. Van Nostrand Company Inc.
Hussain, A. I., Anwar, F., Hussain Sherazi, S. T., & Przybylsk, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*, *108*, 986−995.
Jayashree, T., & Subramanyam, C. (1999). Antiaflatoxigenic activity of eugenol is due to inhibition of lipid peroxidation. *Letter Applied Microbiology*, *28*, 179−183.
Kartnig, T., & Simon, B. (1986). Yield and composition of volatile oil of various cultivars of *Ocimum basilicum* L. depending on harvest time. *Gartenbauwissenschaft*, *51*, 223−225.
Kokwaro, J. O. (1980). *Medicinal plants of East Africa* (pp. 111). Nairobi, Kenia: East Africa Publishing Bureau.
Kurita, N., & Koike, S. (1983). Synergetic antimicrobial effect of ethanol, sodium chloride, acetic acid and essential oils components. *Agricultural Biology and Chemistry*, *47*, 67−75.
Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G. J. E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*, *91*, 453−462.
Marasas, W. F. O., Kriek, N. P., Fincham, J. E., & Van Rensburg, S. J. (1984). Primary liver cancer and oesophageal basal cell hyperplasia in rat caused by *Fusarium moniliforme*. *International Journal of Cancer*, *34*, 383−387.
Nelson, P. E., Desjardins, A. E., & Plattner, R. D. (1993). Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry, and significance. *Annual review of Phytopathology*, *31*, 233−252.
Pino, J., Roncal, E., Rosado, A., & Goiie, I. (1994). The essential oil of *Ocimum basilicum* L. from Cuba. *Journal of Essential Oil Research*, *6*, 89−90.
Pinto, E., Ribeiro Salgueiro, L., Cavaleiro, C., Palmeira, A., & Gonçalves, M. (2007). In vitro susceptibility of some species of yeasts and filamentous fungi to essential oils of *Salvia officinalis*. *Industrial Crops and Products*, *26*, 135−141.
Provine, H., & Hadley, S. (2000). Preliminary evaluation of a semisolid agar antifungal susceptibility test for yeasts and molds. *Journal of Clinical Microbiology*, *38*, 537−541.
Reddy, K., Reddy, C., & Muralidharan, K. (2009). Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. *Food Control*, *20*, 173−178.
Serrano, M., Martínez-Romero, D., Castillo, S., Guillén, F., & Valero, D. (2005). The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative Food Science and Emerging Technology*, *6*, 115−123.
Shephard, G. S., Sydenham, E. W., Thiel, P. G., & Gelderblom, W. C. A. (1990). Quantitative determination of fumonisins B1 and B2 by high-performance liquid chromatography with fluorescence detection. *Journal of Liquid Chromatography*, *13*, 2077−2087.
Theumer, M. G., Lopez, A. G., Masih, D. T., Chulze, S. N., & Rubinstein, H. R. (2002). Immunobiological effects of fumonisin B1 in experimental subchronic mycotoxicoses in rats. *Clinical and Diagnostic Laboratory Immunology*, *9*, 149−155.
Thoppil, J., Tajo, A., & Minija, J. (1998). Antibacterial and antifungal activity of four varieties of *Ocimum basilicum*. *Fitoterapia*, *69*, 191−192.
Torres, A., Ramirez, M., Arroy, M., Chulze, S., & Magan, N. (2003). Potential use of antioxidants for control of growth and fumonisin production by Fusarium verticillioides and F. proliferatum on whole maize grain. *International Journal of Food Microbiology*, *83*, 319−324.
Van Duong, N. (1993). *Medicinal plants of Vietnam, Cambodia and Laos* (pp. 211−212). Hanoi, Vietnam: Mekong Printing.
Voss, K. A., Plattner, R. D., Bacon, C. W., & Norred, W. P. (1990). Comparative studies of hepatotoxicity and fumonisin B1 and B2 content of water and chloroform/methanol extracts of *Fusarium moniliforme* strain MRC 826 culture material. *Mycopathologia*, *112*, 81−92.
Yu, X., & Cheng, B. (1986). Analysis of the chemical constituents of *Ocimum gratissimum* variety *suave*. *Yunnan Zhiwu Yanjiu*, *8*, 171−174.