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Effect of Essential Oils on the Growth of *Fusarium verticillioides* and Fumonisin Contamination in Corn

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Essential oils extracted by hydrodistillation from local plants in Benin, western Africa, and oil from seeds of the neem tree (*Azadirachta indica*) were evaluated in vitro and in vivo for their efficacy against *Fusarium verticillioides* infection and fumonisin contamination. Fumonisin in corn was quantified using a fluorometer and the Vicam method. Oils from *Cymbopogon citratus*, *Ocimum basilicum*, and *Ocimum gratissimum* were the most effective in vitro, completely inhibiting the growth of *F. verticillioides* at lower concentrations over 21 days of incubation. These oils reduced the incidence of *F. verticillioides* in corn and totally inhibited fungal growth at concentrations of 8, 6.4, and 4.8 μL/g, respectively, over 21 days. At the concentration of 4.8 μL/g, these oils did not affect significantly fumonisin production. However, a marked reduction of fumonisin level was observed in corn stored in closed conditions. The oils adversely affected kernel germination at 4.8 μL/g and therefore cannot be recommended for controlling *F. verticillioides* on stored corn used as seeds, when used at this concentration. The oil of neem seeds showed no inhibitory effect but rather accelerated the growth of *F. verticillioides*.

KEYWORDS: Corn; *Fusarium verticillioides*; fumonisin; essential oils; *Cymbopogon citratus*; *Ocimum basilicum*; *Ocimum gratissimum*

INTRODUCTION

Essential oils are mixtures of different volatile aromatic compounds extracted by stream or hydrodistillation from plants. Since the discovery of their antifungal and antimicrobial properties, preparations of essential oils have been applied in pharmacology, medical microbiology, phytopathology, and food preservation (*1*). The use of essential oils to control postharvest fungi and pests is gaining attention because of the increasing public concern over the level of pesticide residues in foods (*2*). Essential oils are also less likely to be associated with the development of resistance than is the case with fungicides and are less hazardous to the environment and human health than synthetic pesticides (*3*).

Essential oils have been used against pre- and postharvest fungi, much more against *Aspergillus fiavus* Link (*2*, *4*−*9*) than against *Fusarium verticillioides* (Sacc.) Nirenberg (previously known as *F. moniliforme* Sheldon) (*6*, *10*, *11*). This fungus is, however, one of the predominant pathogens associated with corn worldwide (*12*). Not only does it cause corn ear and kernel rot, it is also known to be the most important producer of fumonisin mycotoxins (*13*).

Fumonisins are a group of economically important mycotoxins found in corn and corn-based products (*14*). They have been found to be associated with several animal diseases such as leukoencephalomalacia in horses (*15*) and pulmonary edema in pigs (*16*). With respect to humans, their occurrence in corn has been associated with high incidences of esophageal cancer (*17*, *18*) and liver cancer (*19*).

In light of the concern surrounding exposure to fumonisin, essential oils were tried successfully against fumonisin production, such as thyme and anise oils (*6*) and clove oil (*11*). The present study was undertaken to test the antifungal activity of a range of essential oils extracted from plants commonly encountered in Benin, in western Africa, against the growth of *F. verticillioides* and subsequent fumonisin production in corn.

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Table 1. Plants and Plant Parts Used for Oil Extraction and Mean Oil Yield

scientific name of plants	common English name	registration no.[a]	plant parts	essential oil yield (%) range	essential oil yield (%) mean
Cymbopogon citratus	lemongrass, citronella	AAC 173 HNB	leaves	0.5–1.3	0.9
Ocimum basilicum	basil, sweet basil	AAC 175 HNB	leaves	0.2–1.2	0.7
Ocimum gratissimum	African basil	AAC 176 HNB	leaves	0.2–1.2	0.7
Lantana camara	lantana, shrub verbena	AAC 174 HNB	leaves	0.2–1.2	0.7
Eucalyptus citriodora	lemon eucalyptus	AAC 181 HNB	leaves	2.8–6.0	4.4
Clausena anisata	horsewood, clausena	AAC 177 HNB	leaves	1.2–2.0	1.6
Melaleuca quinquenervia	niaouli	AAC 179 HNB	leaves	1.2–3.6	2.4
Xylopia aethiopica	African pepper	AAC 180 HNB	fruits (pods + seeds)	3.0–6.0	4.5
Azadirachta indica	neem	AAC 178 HNB	seeds		

[a] These numbers are the registration numbers given to each specimen or part of plant when they were deposited at the National Herbarium of Benin, University of Abomey-Calavi, Benin.

MATERIALS AND METHODS

Oil Extraction. Oils were extracted from parts of nine plant species collected during the dry season from different areas of southern Benin **(Table 1**). Dry seeds of neem and pods of *Xylopia aethiopica* were purchased from a local market. Voucher specimens of the plants were deposited at the National Herbarium of Benin, University of Abomey-Calavi, Benin, where they were numbered **(Table 1**). After collection, the leaves were dried for 4 days in the laboratory at room temperature (25−30 °C) before oil extraction. The essential oils were extracted by hydrodistillation for 3 h using a Clevenger apparatus (*20*). The oil from neem seeds was extracted using the Soxhlet method, with hexane as solvent. Oil yields obtained after extraction are shown in **Table 1**. The oils were dried with anhydrous sodium sulfate and stored at 4 °C before use.

Chemical Analyses of the Extracted Oils. The essential oils were analyzed by capillary gas chromatography (GC) coupled with flame ionization detection (FID) as well as mass spectrometric detection (GC-MS). GC analysis was carried out on a Trace GC ThermoQuest system equipped with a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The oven temperature was maintained at 50 °C for 5 min and then programmed to 300 °C at a rate of 5 °C/min. The injector (splitless) and FID temperatures were maintained at 240 and 250 °C, respectively. The carrier gas was hydrogen with a flow rate of 35 mL/min, and the combustion gas was dry air with a flow rate of 350 mL/min.

GC-MS analysis was performed using a Hewlett-Packard 5970 GC fitted with a DB-1 column (25 m × 0.23 mm i.d.). An electron ionization system was used with ionization energy of 70 eV. Helium was used as the carrier gas at a flow rate of 0.9 mL/min. The column temperature was initially kept at 50 °C for 5 min, then increased to 180 °C at a rate of 3 °C/min, and finally increased to 250 °C and maintained at this temperature for 5 min. Samples of 1 μL of oil diluted in 5% pentane were injected. Compounds in oil were identified on the basis of both their Kovats retention indices and mass spectral fragmentation, in comparison with those observed for standards. Some were identified by using literature data (*21*) and the laboratory data bank.

Fungal Culture. The culture of *F. verticillioides* used in this study was obtained from a sample of maize collected during a survey of farmers' stores in southern Benin in 2002. A culture of this fungus has been deposited in the culture collection of the PROMEC Unit of the Medical Research Council (MRC), Tygerberg, South Africa as MRC 8515. The maintenance medium used during preservation was Difco PDA.

Antifungal Activity of Oils in Vitro. The oils were tested at different concentrations of 0.7, 1.3, 2.0, 2.7, 3.3, 4.0, 4.7, 5.3, 6.0, 6.7, and 13.3 μL/mL of PDA to control growth of *F. verticillioides.* These concentrations were obtained by mixing 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, and 200 μL of each oil with 15 mL of melted sterile PDA, respectively. The mixture of oil (at each concentration) and PDA was poured into 10 Petri dishes (90 mm diameter). The Petri dishes were inoculated by placing, at the center of each of them, a 5 mm diameter disk taken from the rim (the actively growing zone) of a 7-day-old *F. verticillioides* culture. The inoculated dishes were incubated for 21 days at 25 °C exposed to a 12:12 h light/dark regimen. Ten Petri dishes containing PDA with no oil were inoculated to serve as controls. Fungal growth was assessed by measuring colony diameters along two lines at right angles to each other after 7, 14, and 21 days.

Antifungal Activity of Oils in Vivo. One hundred autoclaved corn kernels (25 g of kernels) were artificially inoculated with 1 mL of a conidial suspension of *F. verticillioides* (1×10^6 conidia/mL). The conidial suspension was prepared by adding 5 mL of sterile distilled water to a 7-day-old culture. The culture was superficially scraped to free the conidia from conidiophores, and the conidial suspension was filtered through a muslin cloth into 100 mL flasks. The inoculated kernels were treated with oils from *Cymbopogon citratus*, *Ocimum basilicum*, and *Ocimum gratissimum*, which were most effective against *F. verticillioides* in the in vitro assay, at concentrations of 0.8, 1.6, 2.4, 3.2, 4.0, 4.8, 5.6, 6.4, 7.2, and 8.0 μL/g These concentrations were obtained by adding 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 μL of each oil to 25 g of corn kernels (100 kernels). Before treatment, each volume of oil was diluted in 100 μL of 95% ethanol to enhance their solubility and added to 1 mL of sterile distilled water. The kernels were allowed to soak in this mixture for 10 min, dried for ~15 min, and plated onto PDA in Petri dishes. Five kernels were plated on each of 10 Petri dishes per oil concentration with two replications. The Petri dishes were incubated at 25 °C exposed to a 12:12 h light/dark regimen. Antifungal activity of oils was assessed at 7, 14, and 21 days by counting the number of kernels giving rise to *F. verticillioides* colonies in each Petri dish and calculating the incidence of the fungus (percentage of infected kernels per oil concentration). There were two controls: corn kernels inoculated with *F. verticillioides* and treated with a solution of 100 μL of 95% ethanol plus 1 mL of sterile distilled water, and corn kernels inoculated with *F. verticillioides* with no further treatment.

Effect of Essential Oils on Fumonisin Production. Three replicate samples of 100 g of corn kernels were artificially inoculated with *F. verticillioides* and separately treated, as described for the in vivo test, with the oils from *Cy. citratus*, *O. basilicum*, and *O. gratissimum*, at 4.8 μL/g of oil concentration. The kernels were stored for 6 weeks in a laboratory at room temperature (25−30 °C), either in open 200 mL flasks or in closed 200 mL flasks with screw-top lids. Total fumonisin content was assessed in kernels at 0, 3, and 6 weeks of storage with a fluorometer using the Vicam method (*22*). *A* mixture of ground kernels (50 g) with 5 g of sodium chloride and 100 mL of methanol/water (80:20) was blended at high speed for 1 min and filtered through a fluted filter paper. The extract (10 mL) was diluted with 40 mL of phosphate-buffered saline (PBS)/0.1% Tween-20 wash buffer and filtered through a 1.0 μm microfiber filter. The diluted extract was passed through the immunoaffinity column, which was washed with 10 mL of PBS/0.1% Tween-20 wash buffer followed by 10 mL of PBS. Fumonisins were eluted from the column with 1 mL of HPLC grade methanol. A mixture of developer A and developer B (1 mL) was added to the eluate and collected in a cuvette for fumonisin measurement.

Effect of Essential Oils on Seed Germination. This test was performed to determine whether oils at the concentration of 4.8 μL/g

have an effect on germination of corn kernels. Four replicates of 25 kernels (25 kernels weighed ~6.25 g) were separately treated with the oils from *Cy. citratus*, *O. basilicum*, and *O. gratissimum* at the concentration of 4.8 μL/g. This concentration was obtained by using 30 μL of oil for treating 25 kernels. Before treatment, oil (30 μL) was diluted in 25 μL of 95% ethanol and thoroughly mixed to 25 mL of sterile distilled water, on the basis of the method used by Kritzinger et al. (*7*). The treated kernels were allowed to germinate in Petri dishes on double-layered wetted Whatman no. 1 filter paper. Two controls were used. The first of these consisted of sound corn kernels treated with the same quantities of 95% ethanol and sterile distilled water, whereas the second was sound kernels that received no further treatment. Petri dishes containing five kernels each were incubated at ±25 °C. The percentage of germinated kernels per treatment was determined after 7 days.

Statistical Analyses. Statistical analyses were performed using SPSS for Windows version 10.0 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) and Tukey HSD or Student−Newman−Keuls tests were used to compare the means of the growth diameter of *F. verticillioides*, the means of fungal incidence, the means of total fumonisin levels measured in corn treated with the oils, and the means of percentage of germinated kernels.

RESULTS AND DISCUSSION

Chemical Composition of Essential Oils. GC analysis revealed that several compounds were present in the essential oils, of which monoterpenes were predominant (**Table 2**). Oil from *Cy. citratus* mainly contained citral (neral and geranial) (47%) and myrcene (28%). That from *O. basilicum* was richer in the alcohols linalol (33%), eugenol (22%), and estragol (20%). Oil from *O. gratissimum* predominantly contained *p*-cymene (22%) and its precursor, γ-terpinene (15%), and thymol (17%). The major compounds detected in the oil from *Lantana camara* were the sesquiterpenes β-caryophyllene (32%) and α-humulene (11%). Oil from *Eucalyptus citriodora* contained mainly citronellal (66%) along with a small amount of the alcohol citronnellol (12%). Estragol was the major compound identified in *Clausena anisata* oil (up to 93%), whereas that found in *Melaleuca quinquenervia* oil was the oxide 1,8-cineole (51%). Oil from *X. aethiopica* mainly contained the terpinoid sabinene (30%).

The oil from basil (*O. basilicum*) used in this study contained more eugenol (22%) and estragol (20%) than the European French basil, which is likely to contain rather more cineole and linalol (*23*). Moreover, the chemotype of *Cy. citratus* in this study contained less of the aldehyde geranial (27%) than that grown in India (40−62%), and the fact that it is richer in myrcene (28%) is likely to make it highly susceptible to oxidative polymerization, therefore shortening its shelf life (*24*).

Antifungal Activities of Oils. With the exception of *M. quinquenervia* and *X. aethiopica*, all of the essential oils tested were found to significantly affect the growth of *F. verticillioides* after 7 days of incubation ($p < 0.01$) (data not shown). They fully inhibited the growth of *F. verticillioides* at concentrations of <2.7 μL/mL. This inhibitory effect, however, did not last in most cases and significantly decreased with time ($p < 0.01$) as the fungus started to grow after 7 days of incubation. Only the oils from *Cy. citratus*, *O. basilicum*, and *O. gratissimum* remained effective over 21 days, with minimal inhibitory concentrations (MIC) of 1.3, 1.3, and 2.0 μL/mL, respectively (**Table 3**). Soliman and Badeaa (*6*) also found the oil from *O. basilicum* to significantly reduce the growth of pathogenic fungi including *F. verticillioides*. Owolade et al. (*25*) tested five aqueous plant extracts against *F. verticillioides* and found that from *O. gratissimum* to completely inhibit growth of the fungus. These results thus add further support for the view that essential oils could be effectively used for controlling pathogenic fungi.

Table 2. Major Components in Tested Plant Oils

oil	total no. of identified compounds	% oxygenated compounds	major components	%	Kovats retention index
Cy. citratus	16	61	myrcene	28	995
			neral (citral B)	20	1254
			geranial (citral A)	27	1283
			geraniol	4	1268
O. basilicum	24	87	linalol	33	1098
			eugenol	22	1356
			estragol	20	1193
			trans-α-bergamotene	5	1431
			terpinen-4-ol	4	1176
O. gratissimum	29	27	*p*-cymene	22	1030
			γ-terpinene	15	1063
			thymol	17	1303
			α-thujene	5	930
			myrcene	4	995
			caryophyllene oxide	3	1591
			1,8-cineole	2	1045
L. camara	23	37	β-caryophyllene	32	1442
			α-humulene	11	1476
			γ-cadinene	7	1519
			1,8-cineole	6	1051
			spathulenol	6	1580
			γ-*epi*-eudesmol	5	1606
			β-eudesmol	4	1647
			α-phellandrene	2	1042
E. citriodora	15	86	citronnellal	66	1162
			citronnellol	12	1241
			citronnellyl acetate	4	1362
			isopulegol	3	1170
Cl. anisata	17	83	estragol	93	1207
M. quinquenervia	26	77	1,8-cineole	51	1045
			α-terpineol	11	1205
			viridiflorol	10	1611
			spathulenol	3	1574
X. aethiopica	31	20	sabinene	30	978
			β-pinene	8	981
			germacrene D	8	1492
			terpinen-4-ol	7	1191
			1,8-cineole	6	1048
			linalol	2	1116
			α-terpineol	2	1205
A. indica					

The inhibitory effect of the oils increased as oil concentration increased (**Table 3**). The oils from *L. camara* and *E. citriodora* gave rise to complete inhibition of fungal growth when their concentrations increased to more than 4.0 and 4.7 μL/mL, respectively. These two oils displayed only moderate inhibition of *F. verticillioides* growth (**Table 3**). Baruah et al. (*10*), testing four essential oils against *F. verticillioides*, also observed that the oil from *E. citriodora* was less effective than the oil from *Cymbopogon* spp. The oils from *Cl. anisata*, *M. quinquenervia*, and *X. aethiopica* exhibited no significant effect except at concentrations >6.0 μL/mL **(Table 3**). Cardwell and Dongo (*26*) found the aqueous plant extract from *X. aethiopica* used alone to have no fungitoxicity. However, combined with the extract from dried seeds of *Piper guineense* Schum. & Thonn, it totally inhibited the growth of several fungi even more effectively than the aqueous extracts from *O. basilicum* and *O. gratissimum*.

The oil from neem did not show any inhibitory effect, but rather accelerated the growth of *F. verticillioides*, confirming the findings of Juglal et al. (*11*). This oil is well-known to be more effective in controlling storage insect pests (*27*). Carpinella et al. (*28*), however, found extracts from parts of *Melia*

Table 3. Effect of Plant Oils on Growth of *F. verticillioides* after 21 Days of Incubation in an in Vitro Test[a]

	mean fungal colony diameter (mm) at concn of										
oil	0.7 μL/mL	1.3 μL/mL	2.0 μL/mL	2.7 μL/mL	3.3 μL/mL	4.0 μL/mL	4.7 μL/mL	5.3 μL/mL	6.0 μL/mL	6.7 μL/mL	13.3 μL/mL
Cy. citratus	5 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
O. basilicum	46 d	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
O. gratissimum	48 c	5 b	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
L. camara	45 b	32 c	28 c	23 c	21 c	0 a	0 a	0 a	0 a	0 a	0 a
E. citriodora	90 e	33 d	13 b	9 b	4 b	3 b	0 a	0 a	0 a	0 a	0 a
Cl. anisata	90 e	77 f	65 e	67 e	42 d	25 c	20 b	16 b	5 b	0 a	0 a
M. quinquenervia	90 e	75 e	69 d	60 d	42 d	36 d	32 c	28 c	20 c	0 a	0 a
X. aethiopica	90 e	90 g	90 f	90 f	77 e	75 e	65 d	60 d	55 d	42 b	8 b
A. indica	90 e	90 g	90 f	90 f	90 f	90 f	90 e	90 e	90 e	90 c	90 c
untreated control	90 e	90 g	90 f	90 f	90 f	90 f	90 e	90 e	90 e	90 c	90 c

[a] Means within a column followed by the same letter are not significantly different ($P > 0.05$) according to the Tukey HSD test.

Table 4. Minimum Inhibitory Concentration (MIC) of Plant Oils on *F. verticillioides* at 7, 14, and 21 Days of Incubation in in Vitro and in Vivo Tests

	MIC[a] in in vitro test (μL/mL)			MIC[a] in in vivo test (μL/g)		
oil	after 7 days	after 14 days	after 21 days	after 7 days	after 14 days	after 21 days
Cy. citratus	1.3	1.3	1.3	8.0	8.0	>8.0
O. basilicum	0.7	1.3	1.3	6.4	6.4	6.4
O. gratissimum	0.7	1.3	2.0	4.8	4.8	4.8
L. camara	1.3	4.0	4.0	not tested[b]	not tested	not tested
E. citriodora	2.7	4.7	4.7	not tested	not tested	not tested
Cl. anisata	1.3	4.0	6.7	not tested	not tested	not tested
M. quinquenervia	4.0	6.7	6.7	not tested	not tested	not tested
X. aethiopica	13.3	>13.3	>13.3	not tested	not tested	not tested
A. indica	not effective	not effective	not effective	not tested	not tested	not tested

[a] Minimum concentration of oil at which there is no fungal growth. [b] The in vivo test was performed with *Cy. citratus*, *O. basilicum*, and *O. gratissimum* only.

azedarach L., a tree close to the neem, both belonging to the Meliaceae family, to show fungicidal activity on *F. verticillioides*.

The in vivo test showed that the oils from *Cy. citratus*, *O. basilicum*, and *O. gratissimum* all reduced significantly the incidence of *F. verticillioides* in artificially inoculated corn, compared to the controls ($p < 0.01$). At 4.0 μL/g, these oils significantly reduced the fungal incidence to <20%, and complete inhibition occurred at 8.0 μL/g for *Cy. citratus*, at 6.4 μL/g for *O. basilicum*, and at 4.8 μL/g for *O. gratissimum*, over 21 days **(Table 4**; **Figure 1**).

The antifungal activities of these oils might be attributable to the main compounds that they contain. Some of the oils contained several compounds found in previous studies to have biological activities, such as eugenol (*11*, *29*), *p*-cymene (*6*), thymol (*6*, *30*), linalol (*30*), and caryophyllene oxide (*1*). Citral and myrcene are likely to be the active components in the oil from *Cy. citratus*, linalol and eugenol in *O. basilicum* oil, and *p*-cymene and its precursors γ-terpinene and thymol in *O. gratissimum* oil. On the other hand, the lower efficacy of *M. quinquenervia* oil probably was due to 1,8-cineole (51%) as demonstrated in other studies (*5*, *11*). *Cl. anisata* oil exhibited no significant action probably due to estragol (93%). It is more likely that synergistic or antagonistic actions of the components occurred. The *O. basilicum* oil used in this study would be more active against *F. verticillioides* if it did not contain 20% of estragol, which showed ineffective action in *Cl. anisata*.

Effect of Essential Oils on Fumonisin Production. At the concentration of 4.8 μL/g, the oils from *Cy. citratus*, *O. gratissimum*, and *O. basilicum* did not affect significantly fumonisin production in artificially inoculated corn during storage, as there were no significant differences between the treatments and the control ($p > 0.05$). A reduction of the toxin level, up to 60%, however, was observed **(Figure 2)**. Juglal et al. (*11*) found that 2 μL/mL clove oil resulted in a 78% reduction of fumonisin B_1 levels. The effect of essential oils on toxin production would depend on their concentration. Soliman and Badeaa (*6*), testing oils from thyme, anise, cinnamon, and spearmint against fumonisin production, observed a gradual increase in inhibition due to increased concentration of the oils. It is likely, therefore, that the oils from *Cy. citratus*, *O. gratissimum*, and *O. basilicum* are more effective on fumonisin production at concentrations >4.8 μL/g.

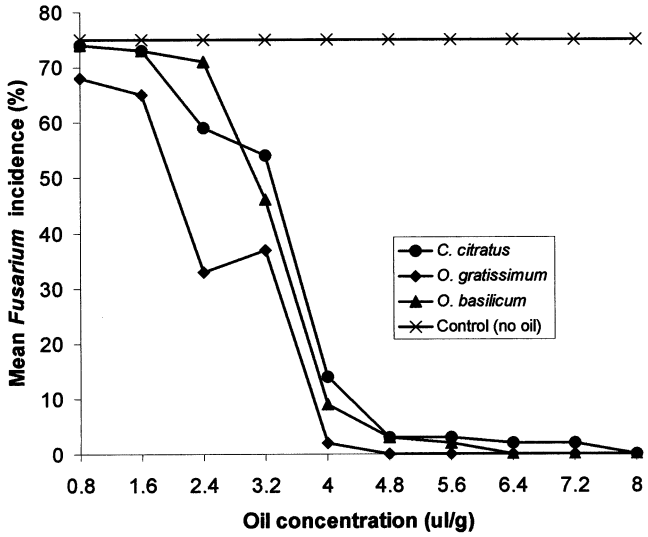

Figure 1. Mean incidence of *F. verticillioides* at different concentrations of essential oils after 21 days of incubation of artificially inoculated corn kernels. The line representing the treatment control + ethanol does not appear in the figure; it is confounded with that of the control (no oil) as these two treatments gave the same results.

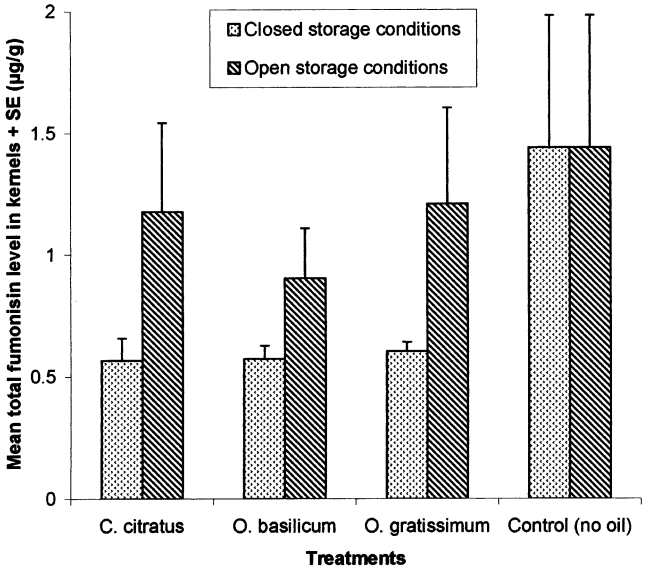

Figure 2. Effect of essential oils on fumonisin production in artificially inoculated corn kernels stored in closed and open conditions during the storage period. The value of fumonisin level for each treatment is the mean of fumonisin levels detected at 0, 3, and 6 weeks of storage. SE = standard error of the mean. The line representing the treatment control + ethanol does not appear in the figure; it is confounded with that of the control (no oil) as these two treatments gave the same results.

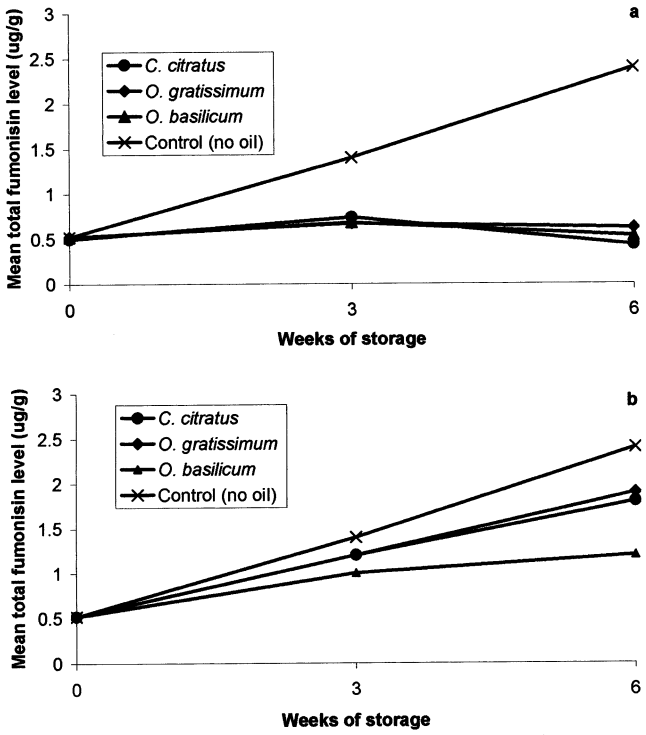

Figure 3. Effect of essential oils on fumonisin production in artificially inoculated corn kernels stored in closed (**a**) and open conditions (**b**) at 0, 3, and 6 weeks of storage.

The reduction observed was more marked when corn was stored in closed conditions than in open storage conditions (**Figure 3**). Fumonisin level was maintained at very low levels (~0.7 μg/g) throughout the 6 week storage period in treated corn stored in closed conditions (**Figure 3a**). In open storage conditions, the level increased with time in all cases (**Figure 3b**). The oils consist of volatile compounds that are more likely to diffuse rapidly in air when the storage container is open. In such open storage conditions, therefore, they would be ineffective in controlling fungal growth and affecting fumonisin production. These findings confirm evidence that the use of essential oils ensures more safety to the consumer as there is an increasing demand for food commodities free of toxins.

Table 5. Effect of Essential Oils on Germination of Corn Kernels[a]

treatment	% germinated kernels
maize treated with *Cy. citratus*	34 a
maize treated with *O. basilicum*	57 ab
maize treated with *O. gratissimum*	44 b
maize treated with 95% ethanol only	77 c
maize not treated	92 c

[a] Each value is a mean percentage of four replicates of 25 kernels. Means followed by the same letter are not significantly different ($P > 0.05$) according to the Student–Newman–Keuls test.

Effect of Essential Oils on Kernel Germination. At the concentration of 4.8 μL/g, the oils from *Cy. citratus*, *O. gratissimum*, and *O. basilicum* adversely affected significantly the germination of kernels ($p < 0.01$) **(Table 5)**. They drastically reduced germination of treated kernels to a nonacceptable level (<60%). *Cy. citratus* and *O. gratissimum* oils exhibited more harmful effects on germination than *O. basilicum* oil. The oils probably killed the embryos, which consequently could not grow. The 95% ethanol did not affect significantly the kernel germination compared to the control ($p > 0.05$) **(Table 5)**. These findings suggest that, at the concentration of 4.8 μL/g, the three oils can be recommended only for treatment of kernels intended for human and animal consumption, not for treatment of kernels to be used as seeds.

This study has demonstrated that essential oils, already commonly used in many parts of the world including Africa for medical purposes, can also serve as alternative means to reduce the growth of *F. verticillioides* in stored corn. Oils from *Cy. citratus*, *O. basilicum*, and *O. gratissimum* proved to be highly effective and can be recommended to small-scale farmers for corn stored in closed conditions. Despite their efficacy and usefulness, the use of the essential oils is, however, limited by several factors. The plants from which they are extracted are being progressively destroyed by extensive agriculture, particularly in Africa. Moreover, most of the current extraction procedures are complicated and costly, particularly to produce sufficient amounts of oil to render their utilization economical. There is an urgent need to protect the plants that produce essential oils with potent fungicidal activity and to promote their cultivation. Research must also continue to find extraction procedures that are simple, inexpensive, and easily performed on small-scale farms.

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