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Research Article
Evaluation of Protective Impact of Algerian *Cuminum cyminum* L. and *Coriandrum sativum* L. Essential Oils on *Aspergillus flavus* Growth and Aflatoxin B_1 Production

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Abstract

Background and Objective: Aflatoxin B_1 (AFB_1) is a highly toxic and carcinogenic metabolite produced by *Aspergillus* species on food and agricultural commodities. The aim of this investigation was to evaluate the inhibition of growth *Aspergillus flavus* E73 (*A. flavus* E73) and AFB_1 production by *Cuminum cyminum* L. (*C. cyminum* L.) and *Coriandrum sativum* L. (*C. sativum* L.) essential oils (EOs) as well their antioxidant and phytotoxicity activities. **Methodology:** The *C. cyminum* L. and *C. sativum* L. EOs were extracted by hydrodistillation. The chemical profile of EOs was identified by GC-MS, antifungal activity was assessed by poisoned food technique and in term Minimal Inhibitory Concentration (MIC) and minimal fungicidal concentration (MFC) and antiaflatoxin effect by broth medium. The antioxidant activity of EOs was determined by DPPH free radical scavenging assay, β-carotene bleaching test and total phenolic content by Folin-Ciocalteu. Phytotoxicity of *C. cyminum* L. and *C. sativum* L. EOs were determined for varieties of wheat. The results were analyzed by analysis of variance (one way ANOVA). **Results:** The GS/MS analysis showed that the major components of *C. cyminum* L. EO were cuminaldehyde (65.98%), o-cymene (18.40%) and *C. sativum* L. EO was mainly consisted of linalool (78.86%). The results showed that both the EOs could inhibit the growth of *A. flavus* E73 in the range of 24.27-84.90% for *C. cyminum* and 15.09-65.00% for *C. sativum*. During antiaflatoxin investigation, the oils exhibited noticeable inhibition on dry mycelium weight and synthesis of AFB_1 by *A. flavus* E73. EOs of *C. cyminum* L. and *C. sativum* L. revealed complete inhibition of AFB_1 at 1.25 and 1.5 mg mL^{-1}, respectively. EOs exhibited inhibitory influence against some fungi. The IC_{50} values of *C. cyminum* L. and *C. sativum* L. EOs were 494.93 and 756.43 μg mL^{-1}, respectively, while, β-carotene/linoleic acid bleaching was 47.68 and 29.29% , respectively. Total phenolic content of *C. cyminum* L. and *C. sativum* L. were 10.66 and 6.2 μg mg^{-1}. Additionally, the EOs were non-phytotoxic on the two verities of wheat seeds. **Conclusion:** The *C. cyminum* L. and *C. sativum* L EOs could be good alternative to protect foods.

Key words: *Cuminum cyminum*, *Coriandrum sativum*, *Aspergillus flavus* E73, aflatoxin B_1, antioxidant, phytotoxicity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The plants are a promising alternative because plants produce a variety of components. Many of plants are generally recognized as safe (GRAS) by the Unites States Food and Drug Administration (FDA). Essential oils (EOs) are natural, volatile, complex plant compounds[1] which can be obtained from different parts of plant such as flowers, seeds, leaves, bark, herbs, fruits and roots by expression, enfleurage, extraction and method of steam distillation. Some EOs appear as a promising approach for inhibition of aflatoxin production which are synthesized by *A. flavus* group species. The most important fungi capable to produce the aflatoxins are *A. flavus*, *A. parasiticus*[2]. Aflatoxins affect cereals, oil seeds, nuts, dry fruits, spices, legumes, fruits, milk and milk derivates[3-4]. It has been reported that consuming food contaminated with aflatoxins especially AFB_1 can cause hepatic carcinoma and other serious diseases vis teratogenicity, immunosuppression and mutagenicity for human beings and other livestock[5]. Another attributes have been revealed by EOs in their antioxidant activity as well as non phytotoxic.

Cumin (*Cuminum cyminum* L.) is herbaceous plant from the Apiaceae family, cultivated basically in Saudi Arabia, India and China[6]. Cumin considered as the second spice after black pepper[7]. Cumin seeds are used in cuisines of many countries such as India, Pakistan, North Africa, Srilanka, Cuba and Mexico[8]. Cumin seeds are used in traditional medicine to treat diseases as toothache, dyspepsia, diarrhea, epilepsy and jaundice[9]. Cumin seeds are also reported to have antioxidant and antimicrobial activity.

Coriander (*Coriandrum sativum* L.) is a plant belonging to the Umbelliferae family. It has various uses, in flavouring, perfumes and cosmetic products. In traditional medicine, *C. sativum* L. have been recommended for dyspepsia, loss of appetite, convulsion and insomnia[10]. It has been proved that *C. sativum* L. possesses antimicrobial and antioxidant activities.

The EOs of *C. cyminum* L. and *C. sativum* L. are consisted of different amounts and volatile components. Chemotypes have been reported for both plants which can be affected by various parameters such as region, environmental conditions, age of plant, the season and the method of extraction.

The study was undertaken to investigate the chemical composition of the EOs from *C. cyminum* L. and *C. sativum* L. and to evaluate their antifungal, antiaflatoxin, antioxidant activity and phytotoxicity.

MATERIALS AND METHODS

Plant material: Essential oils (EOs) were isolated from seeds of *C. cyminum* and *C. sativum* L. collected from the Garden of Reghaia, Algiers, Algeria in 2015. The identification of the two species was firstly given based on their morphological appearances and then confirmed by Doctor Mahdid Mouhamed of Laboratory of Vegetal Ecophysiology of Biology, Department in Normal High School, Kouba, Algiers, Algeria.

Extraction of essential oils: Two hundred grams of dried seeds was subjected to hydrodistillation in Clevenger's apparatus for 3 h. The water traces in the EOs eliminated with anhydrous sodium sulphate (Na_2SO_4). EOs were weighted and stored at 4°C in for further using.

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^{-1}. The column inlet pressure was 8.07 psi. The GC column oven temperature was increased from 60-245°C at 3°C min^{-1}, with a final hold time of 4 min. The 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 and quadrupole, 150°C.

Identification of the volatile compounds: The identification of the volatile compounds was done by comparing the mass spectra (MS) obtained with the NIST electronic databases as well as with the bibliography of Adam[11] in parallel with the use of retention indices (RI) based on series of n-alkane indices (C8-C27) on the capillary column.

Fungal material and preparation of spore inoculum: The aflatoxigenic strain *A. flavus* E73 utilized in this study was obtained from Laboratoire de Biologie des Systèmes Microbiens (LBSM, Kouba, Algeria). Spore inoculum was prepared from the culture of *A. flavus* E73 on Petri dish containing Potato dextrose agar (PDA) for 7 days at 28±2°C and spores were obtained by washing petri dish with 20 mL of 0.1% Tween 80 solution. The number of spores (1×10^6 spores mL^{-1}) was determined using a hemocytometer slide (depth 0.2 mm, 1/400 mm^2)

under a light microscope (Motic: BA210, China). The number of spores of 1×10^6 mL^{-1} was fixed throughout this study.

Antifungal assay: Antifungal activity of *C. cyminum* L. and *C. sativum* L. EOs was tested against the *A. flavus* E73 following the poisoned food technique[12]. Different concentrations of EOs were added to 10 mL PDA at 45-50°C to obtain final concentrations (0.25-2 mg mL^{-1}) and poured into petri dishes. Thereafter, 10 µL of spore suspension was spotted in the centre of each Petri dish and were incubated at 28±2°C for 7 days. The controls were prepared in parallel without EO. Measurements were made daily by taking the average of two perpendicular diameters of each colony. 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:

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

Where:

Da = Average diameter of *A. flavus* E73 growth in the treatment

Db = Average diameter of *A. flavus* E73 growth in the control

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC): The MIC and MFC for *A. flavus* E73 were assessed by broth method of Shukla *et al.*[13]. Different concentrations (0.25-2 mg mL^{-1}) of *C. cyminum* L. and *C. sativum* L. EOs were added to 10 mL SMKY broth medium in test tubes. Tubes with only SMKY (sucrose: 200 g, $MgSO_4.7H_2O$: 0.5 g, KNO_3: 0.3 g, yeast extract: 7 g 1000 mL distilled water) medium (10 mL) used as control. The tubes were inoculated with 10 µL of spore suspension and incubated at 28±2°C for 7 days. The lowest concentration of EOs that did not show any growth of *A. flavus* E73 during 7 days was considered as the MIC. After 7 days, 100 µL from the tubes, where there was no growth, were subcultured on fresh PDA. The lowest concentration of EOs, where no growth reversal carried out during 7 days of incubation was considered as the MFC.

Evaluation of essential oils as aflatoxin B_1 suppressor: According to Mishra *et al.*[14], 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 occurred. For the extraction of AFB_1. The content was filtered (Whatman No. 1) and extracted with 20 mL chloroform (Sigma Aldrich, France). 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) (Silica gel, Fluka, Germany). The development of the chromatograms was carried out in a standard tank (20×20 cm) previously saturated with the solvent system: Toluene: Iso-amyl alcohol: Methanol (90: 32: 2, v/v/v) (Sigma Aldrich, France). After migration, the plates was removed and dried at 60°C for 24 h. AFB_1 were detected by placing the plate in UV transilluminator (360 nm) (CN-6, VILBER LOURMAY, France). The AFB_1 appeared as a blue spot.

The mycelia produced in the liquid cultures were removed and washed on Whatman No. 1. The weight of the mycelium was determined after desiccation at 80°C for 12 h. For the quantification of AFB_1, the blue spots on TLC plates were scraped out, dissolved in 5 mL cold methanol and centrifuged at 2000 rpm (Jouan E76) for 5 min. The absorbance of the supernatant was made using a UV-Visible spectrophotometer (6705 UV/Vis, JENWAY) at 360 nm. The quantity of AFB_1 was calculated according to the formula by Tian *et al.*[15]:

$$AFB_1 \text{ content } (\mu g\ mL^{-1}) = \frac{D\times M}{E\times l}\times 1000$$

Where:

D = Absorbance

M = Molecular weight of aflatoxin (312 g mol^{-1})

E = Molar extinction coefficient (21, 800 L mol^{-1} cm)

I = Path length (1 cm cell was used)

Spectrum of fungitoxicity: The *A. carbonarius*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. tamari*, *A. terreus*, *Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp., were used in this investigation to study the antifungal activity of *C. cyminum* L. and *C. sativum* L. EOs in terms of The MIC and MFC.

Evaluation of antioxidant activity

DPPH free radical scavenging method: Volumes of 50 µL of different concentrations (100, 200, 400, 600, 800 and 1000 µg mL^{-1}) of *C. cyminum* L. and *C. sativum* L. EOs were added to 5 mL of 0.004% (w/v) methanolic solution of DPPH (Sigma Aldrich, France). After incubation in dark at room temperature for 30 min, the absorbance was taken using spectrophotometer (6705 UV/Vis, JENWAY) against a blank at 517 nm[16]. Butylated hydroxytoluene (BHT) (Sigma Aldrich, France) was used as standard. The inhibition percentage of DPPH radical was calculated using the following equation[14]:

$$\text{Percentage inhibition (\% I)} = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100$$

Where:

A_{blank} = Absorbance of the control

A_{sample} = Absorbance of the sample

IC_{50} was calculated from the graph plotting between percentage inhibition and concentration. The IC_{50} value was defined as the quantity of antioxidant necessary to inhibit DPPH radical formation by 50%. The results were expressed as the mean values ±SD.

β-carotene/linoleic acid bleaching method: A solution consisted of 0.5 mg of β-carotene in 1 mL of chloroform, 25 µL of linoleic acid and 200 mg Tween 40 (Sigma Aldrich, France) was prepared. After elimination of chloroform by rotary evaporator at 40°C, 100 mL of distilled water was added and the mixture was agitated. EOs (2 g L^{-1}) were dissolved in Dimethyl Sulfoxide (DMSO) (Sigma Aldrich, France) and then 350 µL were added to 2.5 mL of the above mixture and incubated in water bath at 50°C, for 2 h with blanks[17]. BHT was used as a positive control and DMSO as a negative control. The absorbance was estimated spectrophotometrically at 470 nm and the antioxidant activity (% I) was calculated using the formula:

$$\text{Percentage inhibition (\% I)} = \left(\frac{A_{\beta-carotene\ after\ 2\ h}}{A_{initial\ \beta-carotene}}\right) \times 100$$

Where:

$A_{\beta\text{-carotene after 2 h}}$ = Absorbance of β-carotene after 2 h of the experiments

$A_{initial\ \beta\text{-carotene}}$ = Absorbance of β-carotene at the beginning of the experiments. The results were expressed as the mean values ±SD

Determination of total phenolic content of EOs: Aliquots of 125 µL of EOs in DMSO were dissolved in 500µL of distilled water and 125 µL of Folin-Ciocalteu reagent 10 times diluted (Sigma Aldrich, France). 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. After incubation for 2 h at 25±2°C, the absorbance at 760 nm was measured[18]. The same procedure was also applied to the standard solutions of gallic acid (25-200 µg mL^{-1}).

Total phenolic contents concentration of the EOs was calculated from the regression equation of the curve established with the standard gallic acid and expressed in micrograms of equivalents, gallic acid per milligram of EO. The results were expressed as the mean values ±SD.

Phytotoxicity assay: 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)[19]. After surface-sterilizing 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 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. After determining the number of seeds that germinated, the lengths of the radicle and the plumule were measured. The results were reported as the mean values ±SD.

Statistical analysis: All experiments were repeated three times and the results were analyzed by analysis of variance (one way ANOVA) and the mean values (±SD) were considered significantly different when $p<0.05$ using STATISTICA version 6.

RESULTS AND DISCUSSION

Yield and chemical composition of EOs: The yield of *C. cyminum* and *C. sativum* EOs was 1.08±0.15 and 0.70±0.19%, respectively. The results of present study were in accordance with these of Kedia *et al.*[20], who observed that the yield of *C. cyminum* EO was between 0.9 and 1.2%. Ravi *et al.*[21] reported that *C. sativum* EO yield was 0.82%.

The chemical composition of *C. cyminum* and *C. sativum* EO are presented in Table 1. A total of 16 constituents of *C. cyminum* oil, representing 99.98% of the EO. The major components of EO were cuminaldehyde (65.98%), o-cymene (18.39%), α-methyl-benzene methanol (4.51%), β-pinene (4.38%) and 2-thiophene aldehyde (1.89%). The results of present study differed from previous studies where Algerian *C. cyminum* EO was mainly consisted of cuminaldehyde and the 1-phenyl-1,2-ethanediol[22]. Romeilah *et al.*[23] reported that caryophyllene oxide (6.12%), β-pinene (4.89%), geranyl acetate (4.11%) and β-caryophyllene (3.44%) were the most abundant components in *C. cyminum* EO. Other abundant components in *C. cyminum* were α-pinene (29.1%), 1, 8-cineole (17.9%) and linalool (10.4%)[24]. In another study

Table 1: Chemical composition of *C. cyminum* and *C. sativum* EOs

		Percentage	
Components	RI	*Cuminum cyminum*	*Coriandrum sativum*
α-pinene	929	0.22	2.69
Camphene	945	-	0.26
Limonene	967	-	0.17
Myrcene	974	-	0.36
β-pinene	984	4.38	0.36
2-Vinyl-2,3-dihydro-4H-pyran	1017	0.27	-
o-cymene	1021	18.39	2.65
Sabinene	1025	-	1.06
1,8-cineole	1028	0.23	-
γ-Terpinene	1053	-	3.33
Trans-linalool oxide	1066	-	0.21
Terpinolene	1081	-	0.21
Cis-linalool oxide	1082	-	0.20
Linalool	1101	-	78.86
Camphor	1143	-	2.28
4-t-pentylcyclohexane	1163	0.26	-
Borneol	1172	-	0.69
4-Terpineol	1178	-	0.27
Tricyclo[5.1.0.0(2,8)]octane	1192	0.29	-
β-fenchyl alcohol	1195	-	0.46
Methyl chavicol	1196	0.29	-
Decyl aldehyde	1203	-	0.26
m-Cumenol	1237	0.50	-
Cuminaldehyde	1242	65.98	-
Geraniol	1249	-	1.12
1-Phenyl-1-butanol	1284	0.71	-
α-Methyl- benzene methanol	1288	4.51	-
p-cymen-7-ol	1295	0.63	-
2-Thiophene aldehyde	1330	1.89	-
1-Methyl-2 Phenylcyclopropane	1336	0.73	-
Geranyl acetate	1374	-	4.54
Isomenthol	1390	0.70	-
Total identified		99.98	99.98
Monoterpene hydrocarbons		22.99	11.09
Oxygen-containing monoterpenes		72.84	88.89
Others		4.15	0

RI: Retention indices

of Naeini *et al.*[25], α-pinene (30%), limonene (21%) and 1,8-cineole (18.5%) were the main constituents of *C. cyminum* EO. On the other hand, chemical composition of present study *C. sativum* EO constituted of 19 compounds, representing 99.98% of the EO, linalool (78.86%), geranyl acetate (4.54%), γ-terpinene (3.33%), α-pinene (2.70%), p-cymene (2.65%), camphor (2.28%), geraniol (1.12%) and limonene (1.06%). Zoubiri and Baaliouamer[26] revealed that linalool (73.11%), p-mentha-1,4-dien-7-ol (6.51%), α-pinene (3.41%) and neryl acetate (3.22%) were the main constituents in Algerian *C. sativum* EO. Samojlik *et al.*[27] exhibited 14 chemical constituents in *C. sativum* and its major components were linalool (74.6%), camphor (5.9%), geranyl acetate (4.6%) and p-cymene (4.0%).

Antifungal activity assay: The antifungal activity of *C. cyminum* and *C. sativum* EOs is represented in Table 2. A significant activity ($p<0.05$) has been remarked with increasing the concentration of *C. cyminum* and *C. sativum* EOs. As shown, the growth of *A. flavus* E73 was delayed by 4 days at 1.0 mg mL^{-1} for *C. cyminum* and by 1 day at 1.25 mg mL^{-1} for *C. sativum*. The percentage inhibition of the growth of *A. flavus* E73 was reported in the range of 24.27-84.90% for *C. cyminum* and 15.09-65.00% for *C. sativum*.

The antifungal mechanism of EO components is not completely clear yet. However, their low-molecular weight and highly lipophilicity make them pass easily through membranes and disrupt cell organization of the fungus[28].

C. cyminum EO exhibited good antifungal activity which might be attributed to the dominance of o-cymene and cuminaldehyde. These two volatile compounds have been shown to have strong antifungal activity[29-31]. The monoterpene hydrocarbons, β-pinene might be also

Table 2: Antifungal activity of *C. cyminum* and *C. sativum* EOs on *A. flavus* E73

Treatments (mg mL^{-1})	Diameter of mycelial growth (mm) 2 days	3 days	4 days	5 days	6 days	7 days	Growth inhibition at day 7 (%)
Control	15.00±0.50	25.83±1.25	45.33±1.52	58.50±0.50	77.16±0.76	87.16±1.25	-
C. cyminum (0.25)	9.50±0.50	15.33±2.08	24.16±0.28	36.50±0.50	54.00±1.00	66.00±1.50	24.27
C. cyminum (0.50)	8.50±0.50	13.00±1.50	20.83±0.76	25.16±0.76	43.83±0.76	55.00±1.00	36.89
C. cyminum (0.75)	6.83±0.28	10.00±1.00	16.83±1.04	22.00±2.64	31.00±1.00	37.00±1.00	57.54
C. cyminum (1.0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.66±2.08	13.16±0.76	84.90
C. cyminum (1.25)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00
C. cyminum (1.50)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00
C. cyminum (1.75)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00
C. cyminum (2.0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00
C. sativum (0.25)	11.33±1.52	20.00±0.50	34.66±0.57	50.83±1.04	64.16±0.76	74.00±1.00	15.09
C. sativum (0.50)	8.83±0.76	19.00±0.50	28.83±1.25	47.00±1.00	56.16±0.76	61.16±1.04	29.83
C. sativum (0.75)	8.00±0.50	18.16±0.28	25.50±1.32	38.66±0.57	46.50±1.50	53.83±0.76	38.24
C. sativum (1.0)	7.33±0.28	15.16±1.04	21.50±1.32	35.83±1.25	45.16±0.77	52.83±2.36	39.25
C. sativum (1.25)	0.00±0.00	6.50±0.50	11.00±1.00	16.50±0.50	22.66±1.52	30.50±0.50	65.00
C. sativum (1.50)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00
C. sativum (1.75)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00
C. sativum (2.0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	100.00

Values are Mean±SD (n = 3)

Table 3: Effect of *C. cyminum* and *C. sativum* EOs on dry weight of mycelium and AFB_1 production

Treatments (mg mL^{-1})	Dry weight of mycelium (mg) *Cuminum cyminum*	*Coriandrum sativum*	AFB_1 (µg mL^{-1}) *Cuminum cyminum*	*Coriandrum sativum*
Control	905.33±6.11	905.33±6.11	868.25±16.52	868.25±16.52
0.25	616.33±4.50	716.00±6.55	515.22±28.62	667.88±21.86
0.50	472.00±3.46	585.66±6.02	372.10±14.31	491.37±21.85
0.75	264.33±3.51	460.33±8.96	195.59±8.260	381.65±8.260
1.0	120.00±4.08	425.33±5.03	57.24±14.31	372.10±14.31
1.25	0.00±0.00	265.00±4.58	0.00±0.00	205.06±29.70
1.50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1.75	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values are Mean±SD (n = 3)

involved in the higher antifungal activity of *C. cyminum* EO. According to De Souza *et al.*[32], pinenes showed antibacterial and antifungal activity. Despite that linalool was found as major constituent, *C. sativum* EO showed moderate antifungal activity. Stevic *et al.*[33] reported that linalool was dominant in coriander with moderate to good antifungal activity. Mishra *et al.*[14] indicated that linalool was moderately antifungal against *A. flavus*.

Generally speaking, there is evidence that minor and major components interact in synergistic and antagonistic manner.

The MICs and MFCs of *C. cyminum* and *C. sativum* EOs were evaluated by broth dilution. This method allows to EOs to contact closely with fungal spores during the distribution in the medium[34]. Their study is important to determine the minimum concentration to inhibit fungal growth. The MIC of *C. cyminum* EO against *A. flavus* E73 was found at 1.25 mg mL^{-1} while MIC of *C. sativum* EO was observed at 1.5 mg mL^{-1}. Results obtained from the MIC test were confirmed with MFC tests, where inhibitory activity of *C. cyminum* occurred at a concentration of 1.5 and 2.0 mg mL^{-1} for *C. sativum*.

It has been shown that the MIC of *C. cyminum* and *C. sativum* EOs were higher than that of *Cicuta virosa*[30], *Ocimum sanctum*[35] and *C. cyminum* L.[20]. However, it was found to be lower than *C. sinensis* var. Valencia[36], *C. sativum*[33] and some prevalent organic preservatives such as salicylic acid, BHA, BHT, ascorbic acid and gallic acid[37].

Efficacy of the EOs on dry mycelium weight and aflatoxin B_1 content: AFB_1 is the most toxic compound. As a consequence, an attempt was made to assess the effect of *C. cyminum* and *C. sativum* EOs to inhibit AFB_1 production. Results showed that *C. cyminum* and *C. sativum* EOs can inhibit weight of mycelium and the AFB_1 synthesis (Table 3). As shown, results revealed that the dry weight of mycelium under the influence of *C. cyminum* was between 616.33 and 120 mg at concentrations ranged between 0.25 and 1 mg mL^{-1}

Table 4: Antifungal activity of *C. cyminum* and *C. sativum* EOs against some fungi

	CMI (mg mL^{-1})		CMF (mg mL^{-1})	
Fungal species	*Cuminum cyminum*	*Coriandrum sativum*	*Cuminum cyminum*	*Coriandrum sativum*
A. carbonarius	1.75	1.50	>2.00	>2.00
A. fumigatus	1.00	1.00	1.50	1.75
A. niger	0.50	0.50	1.25	1.50
A. ochraceus	1.00	1.25	1.50	2.00
A. tamari	1.00	1.00	1.50	1.75
A. terreus	1.00	1.25	1.25	2.00
Fusarium sp.	1.50	1.75	2.00	>2.00
Penicillium sp.	1.25	1.00	1.75	1.25
Rhizopus sp.	1.50	2.00	>2.00	>2.00

compared to the control (905.33 mg) ($p<0.05$) and it was proportional to concentrations of the EO supplemented in the SMKY medium. It is clear that *C. cyminum* EO showed inhibition of dry weight of mycelium at all concentrations tested where complete inhibition was occurred at 1.25 mg mL^{-1}. The same results were obtained for *C. sativum* EO, dry weight of mycelium diminished proportionally in concentrations ranging from 0.25-1.25 mg mL^{-1} (716-265 mg) when compared to the control (905.33 mg) ($p<0.05$) and the total inhibition was carried out at 1.5 mg mL^{-1}.

AFB_1 reduction from SMKY broth medium was dependent on the EO concentration. The treatment of *A. flavus* with different concentrations of *C. cyminum* EO caused varying degrees of AFB_1 inhibition. It is apparently that *C. cyminum* EO at concentration of 0.75 mg mL^{-1} and 1 mg mL^{-1} decreased significantly ($p< 0.05$) the quantities of AFB_1 to 195.59 μg mL^{-1} and 57.24 μg mL^{-1}, respectively. The *C. cyminum* EO exhibited complete inhibition of AFB_1 at 1.25 mg mL^{-1}. On the hand, AFB_1 inhibition increased with increasing of *C. sativum* EO concentrations. The EO generated significant inhibition of 381.65, 372.10 and 205.06 μg mL^{-1} at 0.75, 1 and 1.25 mg mL^{-1} in comparison with the control (868.25 μg mL^{-1}) ($p< 0.05$). It should be noted that AFB_1 was inhibited completely at 1.5 mg mL^{-1}.

According to the above results, *C. cyminum* and *C. sativum* EOs inhibited *A. flavus* E73 growth and AFB_1 production at the same concentrations. Present study findings were similar to the ones of Reddy *et al.*[38], who found that *Syzygium aromaticum* inhibited *A. flavus* growth and AFB_1 production at 5 g kg^{-1}. Mishra *et al.*[39] indicated that Jamrosa EO showed both antifungal activity and inhibition of AFB_1 production at 0.4 μL mL^{-1}, but the inhibition of AFB_1 production cannot be completely attributed to reduced fungal growth. There were many studies confirmed this suggestion. Kumar *et al.*[40] reported that *Lantana indica* EO completely inhibited *A. flavus* growth and AFB_1 production at 1.5 and 0.75 μg mL^{-1}, respectively. Similar types of results were obtained by Srivastava *et al.*[41] where *Cinnamomum camphora* (*C. camphora*) and *Alpinia galanga* (*A. galanga*) inhibited *A. flavus* growth at 1000 ppm and the AFB_1 production at 500 ppm for *A. galanga* and 750 ppm for *C. camphora*. In another study performed by Vilela *et al.*[42], the inhibition of AFB_1 required a concentration of *Eucalyptus globulus* EO greater than was for inhibition of *A. flavus* and *A. parasiticus*.

Because of the extramitochondrially biosynthesis of aflatoxins from acetylcoenzyme A during the glucose utilization. Thus, the inhibition of AFB_1 production can be attributed to the inhibition of carbohydrate catabolism in *A. flavus* by acting on some enzymes in order to diminish its capacity of AFB_1 production[15]. Generally, the inhibition mechanism of AFB_1 production is not very clear as has been reported by those authors. So, *C. cyminum* and *C. sativum* EOs may interfere with some steps in the metabolic pathways of the *A. flavus*, which controls AFB_1 biosynthesis.

Spectrum of fungitoxicity: The fungitoxicity of *C. cyminum* and *C. sativum* EOs at concentrations between 0.25 and 2 mg mL^{-1} was tested. Results of antifungal activity of the EOs are shown in Table 4. The *C. cyminum* EO inhibited the growth of most fungi at concentration between 0.5 and 1.75 mg mL^{-1}. The highest concentration of this EO was that for *A. carbonarus* (1.75 mg mL^{-1}) and the lowest was that for *A. niger* (0.5 mg mL^{-1}). It can be clearly seen that *C. cyminum* EO showed slightly lower inhibition compared to *C. sativum* EO which was between 0.5 and 2 mg mL^{-1}, except *A. niger*, *A. fumigates* and *A. tamari*, the inhibition occurred somehow at the same concentration (0.5, 1.0 and 1.5 mg mL^{-1}, respectively). *C. sativum* EO exhibited antifungal activity against *A. carbonarius* and *Penicillium* sp. higher than *C. cyminum* EO with MIC 1.58 and 1.0 mg mL^{-1}, respectively. Additionally, MFC was determined for *C. cyminum* and *C. sativum* EOs. Aligiannis *et al.*[43] demonstrated that antimicrobial activity considered strong when MIC to 0.50 mg mL^{-1}, moderate MIC between 0.6 and 1.5 mg mL^{-1}, weak MIC over 1.5 mg mL^{-1}. From the results presented

Table 5: Antioxidant activity and total phenolic of *C. cyminum* and *C. sativum* EOs

EOs	DPPH (IC_{50}) (μg mL^{-1})	β-carotene/Linoleic acid inhibition (%)	Total phenolic content (μg mg^{-1})
C. cyminum	494.93±8.82	47.68±0.68	10.66±0.90
C. sativum	756.43±12.63	29.29±1.19	6.20±0.91
BHT	306.15±4.49	94.77±1.61	nd

nd: Not determined. Values are Mean±SD (n = 3)

herein, *C. cyminum* and *C. sativum* EOs exhibited strong, moderate and weak activity. It should be noted that MFC values were greater than MIC values where they were between 1.25 and >2 mg mL^{-1} for *C. cyminum* EO, 1.58 and >2 mg mL^{-1} for C. sativum EO.

Many research works has studied the antifungal activity of EOs. Kedia *et al.*[20] found that *C. cyminum* EO was active against fungi such as *Alternaria alternata*, *A. niger*, *A. terreus*, *Mucor* sp., *Rhizopus stolonifer* and *Penicillium* species. Prakash *et al.*[37] reported that *C. sativum* EO exhibited inhibitory effect against *A. niger*, *A. candidus*, *A. terreus*, *A. fumigatus*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Fusarium nivale*, *Penicillium italicum* at concentration ranging between 2 and 3 μg mL^{-1}. Stevic *et al.*[33] tested the antifungal activity of *C. sativum* against some fungi viz., *A. flavus*, *A. niger*, *Alternaria alternata*, 8 species of *Fusarium*, *Penicillium* sp., *Chaetomium* sp., *Gliocladium roseum*, *Curvularia lunata*, *Verticillium dahliae*, *Trichoderma viride*, *Trichothecium roseum*, *Phomopsis* sp., *Phoma* sp. and *Myrothecium verrucaria*. The authors found that the EO could inhibit these fungi at concentration between 0.97 and 5.10 mg mL^{-1}. Other EOs have been also tested for their antifungal activity like lemon, orange, mandarin and grapefruit peels[30], *Bidens pilosa*[44] and cinnamon leaf[45]. Overall, based on the efficient antifungal activity of *C. cyminum* and *C. sativum* EOs, they would use for inhibition of fungal contamination of food and as consequence, used as plant antimicrobial.

Antioxidant activity: During this investigation, two different methods have been used to evaluate the antioxidant activity of *C. cyminum* and *C. sativum* EOs: The DPPH radical scavenging activity and β-carotene/linoleic acid bleaching.

Free radical-scavenging ability of *C. cyminum* and *C. sativum* EOs were measured by the DPPH and the obtained results were compared with the standard BHT, which is an efficient synthetic antioxidant agent in food. The DPPH scavenging activity was presented by IC_{50} value (Table 5). The IC_{50} concentration and the antioxidant capacity have inversely proportional values and *C. sativum* (756.43±12.63 μg mL^{-1}) was established to have the lowest antioxidant capacity while *C. cyminum* (494.93±8.82 μg mL^{-1}) was found to be the richest. However, *C. sativum* and *C. cyminum* EOs exhibited lower antioxidant efficacy than BHT (306.15 ±4.49 μg mL^{-1}) ($p<0.05$). These results were different of the ones of Romeilah *et al.*[23], which reported that the radical scavenging activity of *C. cyminum* and *C. sativum* EOs were 83.59 and 74.72%, respectively at 200 μg mL^{-1}. The IC_{50} (72.3 μg mL^{-1}) of *C. cyminum* was lower than IC_{50} (74.05 μg mL^{-1}) of *C. sativum* EO. Kedia *et al.*[20] reported that *C. cyminum* EO showed strong free radical scavenging activity where its IC_{50} was found to be 0.092 μL mL^{-1}. As well, Prakash *et al.*[37] evaluated the antioxidant activity of *C. sativum*, showing that the IC_{50} value of the EO was 2.90 μL mL^{-1}. This difference in DPPH radical scavenging activity could be explained by difference in the chemical composition of *C. cyminum* and *C. sativum* EOs.

The capacity of *C. cyminum* and *C. sativum* EOs to inhibit lipid peroxidation was tested by the β-carotene/linoleic acid bleaching test. The bleaching mechanism of β-carotene is the result of the formation of hydroperoxides from linoleic acid[46]. Chew *et al.*[47] reported that the antioxidants in the different natural samples can limit β-carotene bleaching.

The oxidation of β-carotene was stopped by EOs of *C. cyminum* and *C. sativum* where values were about of 47.68±0.68 and 29.29±1.19%, respectively, comparable to BHT (94.77±1.61%) ($p<0.05$) (Table 5). Generally, results of β-carotene bleaching were less than those provided by the radical-scavenging activity.

Total phenolic content: Total phenolics were calculated as μg gallic acid equivalent mg^{-1} of EO. *C. cyminum* EO had higher total phenolic content (10.66±0.90 μg mg^{-1}) than *C. sativum* EO (6.2±0.91 μg mg^{-1}) (Table 5). Rebey *et al.*[48] reported that phenolic content of *C. cyminum* EO was 18.32 mg g^{-1}. On the other hand, Prakash *et al.*[37] found that phenolic content of *C. sativum* EO was 4.15μg mg^{-1}.

In comparison with present study results, previous studies showed a significant correlation between the antioxidant activity and total phenolic contents in *C. sativum* [49], herbs, vegetables and fruits[50-51]. However, the antioxidant activity of EOs cannot be related just to phenolics but to other compounds such as monoterpene alcohols, ketones, aldehydes and hydrocarbons.

It has been reported that the oxidative stress and peroxidation cause AFB_1 production by *Aspergillus* spp.

Table 6: Phytotoxic influence of *C. cyminum* and *C. sativum* EOs on seed germination and seedling growth of two varieties of wheat

Samples	Germination (%)	Seedling growth (mm)	
		Radicle	Plumule
HD1220 (Hiddab)			
Control	92.66±3.05	67.70±2.98	57.83±1.30
C. cyminum EO	82.00±2.00	58.53±1.09	46.50±0.26
C. sativum EO	78.66±4.16	58.03±2.74	50.00±2.68
AS 81189 A (Ain Abid)			
Control	90.66±4.16	69.40±2.98	60.30±4.59
C. cyminum EO	83.33±4.16	53.03±2.03	45.40±2.51
C. sativum EO	85.33±5.05	37.53±2.40	22.00±1.83

Values are means (n = 3) ±SD

according to Jayashree and Subramanyam[52], Narasaiah *et al.*[53], Zjalic *et al.*[54] and Kim *et al.*[55]. EOs have been shown to have antioxidant activity in this investigation. Hence, the inhibition of AFB_1 by *C. cyminum* and *C. sativum* EOs may be related to their antioxidant nature.

Phytotoxicity assay: Phytotoxicity of the *C. cyminum* and *C. sativum* EOs were evaluated for assessing their effect on the germination and seedling growth of AS 81189 A (Ain Abid) and HD 1220 (Hiddab). As given in Table 6, *C. cyminum* and *C. sativum* EOs showed no significant effect on the germination of HD1220 (Hiddab) and AS 81189 A (Ain Abid) seeds ($p>0.05$). The length of radicles and plumules in the seeds tested with *C. cyminum* and *C. sativum* EOs was also diminished but the effect of *C. sativum* EO on the length of radicles and plumules of the seeds of AS 81189 A (Ain Abid) tested with *C. sativum* EO was greater than the others. However, EOs did not reveal somehow significant phytotoxicity against the seeds. Hence, *C. cyminum* and *C. sativum* EOs can be recommended for storing food items or sowing purpose.

CONCLUSION

Although *C. cyminum* and *C. sativum* EOs have resulted to possess variety of compounds, antifungal, antiaflatoxin, antioxidant activity, to inhibit fungi growth and show their non phytotoxicity, they require deep evaluation to transform them to more effective and safer preservatives and fungicides in order to decrease using chemical products because nowadays consumer is looking for foods that show more fresh-like and natural characteristics.

SIGNIFICANCE STATEMENT

This study discovers the possible effect of *C. cyminum* and *C. sativum* EOs that can be beneficial to control *A. flavus*, aflatoxin B_1 production and fungal spoilage to assess antioxidant activity and using them as food preservative for enhancement of shelf life of stored food commodities. This study will help the researchers to uncover the critical area of natural alternative to apply in food that many researchers were not able to explore. Thus, a new theory on the relation between antioxidant activity of *C. cyminum* and *C. sativum* EOs and AFB_1 production and possibly other activities, may be arrived at because this point is not completely understood.

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