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Antifungal efficacy of *Cymbopogon citratus L.* essential oil against storage mycoflora isolated from spice cumin

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Abstract
The study deals with the bioactive efficacy of *Cymbopogon citratus* L. essential oil (CCEO), against some storage fungi contaminating stored spice seeds of cumin (*Cuminum cyminum* L.). The average pH and percent moisture content of collected stored seeds of cumin were ranged as 5.31-5.60 and 13.33-14.38% respectively. Stored seeds of cumin were found associated with various storage moulds. During mycological screening, total of 654 fungal isolates were recovered from three different stored samples. The percent occurrence frequency of sample 3 was found highest (36.08%) whereas, sample 1 exhibited the lowest (30.12%). The highest cumulative percent relative density was recorded in *Cladosporium* sp. (21.86%) followed by *Aspergillus niger* (17.80%) and *Aspergillus flavus* (13.14%) while lowest relative density was found in *Aspergillius nidulans* (1.07%) followed by *Aspergillius candidus* (1.37%) and *Aspergillus terreus* (2.14%). The minimum inhibitory concentration (MIC) of CCEO against *Aspergillus flavus* was recorded at 0.6 $mgml^{-1}$. The CCEO also exhibited potent antiaflatoxigenic efficacy and completely checked the aflatoxin B1 production at 0.3 $mgml^{-1}$. The CCEO also showed broad spectrum fungitoxicity against 10 storage fungi recovered during mycological analysis of cumin seeds. The chemotype of CCEO was determined by GC/GC-MS analysis which showed 23 constituents in which E-citral was found to be the major component (60.98%). The prospects of exploitation of CCEO as acceptable plant-based preservative in qualitative as well as quantitative control of biodeterioration of stored cumin have been discussed.

Keywords: *Cymbopogon citratus*; essential oil; antifungal; *Aspergillus flavus*; Citral; *Cuminum cyminum*

Introduction

Spices are important agricultural commodities widely used for their flavour, aroma, and medicinal value [1]. However, during storage and transportation, they are highly susceptible to fungal contamination, leading to quality deterioration, discoloration, and loss of essential oils [2]. Among various spices, cumin (*Cuminum cyminum* L.) holds significant economic and nutritional importance, yet it is prone to colonization by storage fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* species [3]. These fungi not only affect the sensory and nutritional qualities of cumin but also pose serious health risks through the production of mycotoxins, particularly aflatoxins [4]. The use of synthetic fungicides for controlling these contaminants is often limited by their potential toxicity, environmental hazards, and the emergence of resistant fungal strains [5]. Hence, there is an increasing demand for natural, safe, and eco-friendly alternatives for managing fungal contamination in stored spices.

Plant essential oils (EOs) have gained attention as promising natural antifungal agents due to their biodegradability, low toxicity, and broad-spectrum antimicrobial activity [6, 7]. *Cymbopogon citratus* (DC.) Stapf., commonly known as lemongrass, is an aromatic plant whose EO is rich in citral, along with other bioactive constituents such as limonene and myrcene [8, 9]. These compounds exhibit potent antifungal, antioxidant, and antimicrobial activities, making lemongrass oil a potential candidate for use in food preservation [10, 11].

The present study aims to evaluate the antifungal efficacy of *Cymbopogon citratus* essential oil (CCEO) against storage mycoflora isolated from spice cumin. By assessing its inhibitory potential on the growth of dominant fungal contaminants, this study seeks to establish the role of lemongrass oil as a natural fungitoxicant. The outcomes are expected to support the development of sustainable post-harvest management practices that ensure the safety, quality, and extended shelf life of cumin and other spice products.

Materials and Methods

Collection of cumin seed samples and preparation

Three different samples of stored cumin seeds (500 g) were collected from the local market of Gorakhpur, Uttar Pradesh, India, during October-November, 2024. The cumin seed samples were collected in sterilized polythene bags to avoid further contamination. In the laboratory, seed samples were finely ground individually in a common household blender. The blender's cup was rinsed in 90% alcohol before and after grinding the individual sample. The powder was sieved through No. 50 mesh sieve, kept tightly packed in a paper bags and stored at 5ºC for further analysis [6].

PH and Moisture content determination

Aqueous suspensions (1:10; w/v) of powdered spice of *C. cyminum* were prepared and stirred for 5 h, and the pH of suspension was noted using digital pH meter [6].

To determine moisture content, weighed amount (50 g) of samples were dried at 100ºC until their weights remained constant and percent moisture content was calculated following Kumar *et al.* [12].

Moisture content (%) = $(W_1-W_2 / W_1) \times 100$

Where W_1 is the initial weight and W_2 is the final weight after drying.

Mycological screening of collected cumin seed samples

In a conical flask (250 ml), 10 grams of each powdered sample were suspended individually in 100 mL of sterile 0.85% saline solution. The samples were then homogenized on an electric shaker at a constant speed (120 rpm) for 15 minutes. For every sample, three-fold serial dilutions were made independently [6]. The Petri dishes having 10 ml of sterilized potato dextrose agar (PDA) medium were inoculated with 0.5 ml of the dilution (10^{-3}) separately. The inoculated plates were kept at 27±2°C for seven days. The process of counting the colonies started on the third day of incubation. Every mold colony with a unique morphology was recognized after being subcultured on PDA [13, 14].

Detection of aflatoxigenic potential of isolated Aspergillus flavus from cumin seeds

Ten isolates of *Aspergillus flavus* were randomly selected and their aflatoxigenic potency was assessed using SMKY (sucrose, 200.0 g; magnesium sulphate, 0.5 g; potassium nitrate, 0.3 g; yeast extract, 7.0 g; distilled water, 1000 ml; pH, 5.6±0.2) as a broth nutrient medium [12]. Each *A. flavus* isolate was inoculated aseptically into 50 ml of SMKY medium with 1 mL of spore suspension (≈10^6 spores mL^{-1}) in 0.1% Tween-80, and the mixture was then incubated for ten days at 27±2 °C. Following incubation, each flask's contents were filtered (Whatman no. 1). Filtrate of each flask was separately extracted with 40 ml chloroform in a separating funnel. The chloroform extract was separated and evaporated till dryness on water bath at 70 °C. A modified thin layer chromatographic (TLC) technique of Kumar *et al.* [6] was used to determine the aflatoxigenic potency of *A. flavus* using following formula.

$$\text{Aflatoxin } B_1 \text{ content } (\mu g L^{-1}) = \frac{D \times M}{E \times l} \times 1000$$

Where, D-absorbance; M-molecular weight of AFB_1 (312); E-molar extinction coefficient of AFB_1 (21,800); l-path length (1 cm cell was used)

Extraction of Cymbopogon citratus leaf essential oil (CCEO)

C. citratus leaves were collected from the botanical garden, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur for the extraction of EO. Leaves (500 g) were thoroughly washed with distilled water and subjected to Clevenger's hydrodistillation apparatus for three hours. The collected CCEO was dehydrated using sodium sulphate and stored in dark clean glass vial at 4-5°C [15].

GC/GC-MS analysis of CCEO

Gas chromatography (Perkin Elmer Auto XL GC) with a flame ionization detector was used to analyze the CCEO. The following were the GC conditions: column, EQUITY-5 (60m × 0.32mm × 0.25µm) fused silica capillary column; H_2 was the carrier gas; column head pressure was 10 psi; oven temperature program isotherm was 2 min. at 70ºC, gradient was 3ºC/min to 250ºC, isotherm 10 minutes; injection temperature 250ºC; detector temperature 280ºC. Additionally, Perkin Elmer Turbomass GC-MS was used for GC-MS analysis. The GC column's effluent was added straight to the MS source. With ionization energy of 70 eV, spectra were acquired in the EI mode. By comparing the mass spectra and relative retention durations of the compounds with those of genuine reference compounds published in the literature, the compounds were identified [16].

Antifungal and antiaflatoxigenic activity of CCEO

Minimum inhibitory concentration (MIC) and antiaflatoxigenic efficacy of CCEO was determined against *A. flavus* DDUCC-8 using SMKY broth medium. Different concentrations of the CCEO were prepared separately by dissolving their requisite amount in 0.5 ml 5% tween-20 followed by 49.5 ml of SMKY medium. The control sets were kept parallel to the treatment sets without CCEO. The flasks were inoculated aseptically with 1 ml spore suspension (≈10^6 spores/ml) of *A. flavus* DDUCC-8 and incubated at 27±2 °C for 10 days. After incubation, mycelial biomass and aflatoxin B_1 content in broth medium of each flask was determined [6].

Fungitoxic spectrum of CCEO

The spectrum of fungitoxicity of the CCEO was determined at 0.6 $mgmL^{-1}$ (MIC against *A. flavus* DDUCC-8) by the poisoned food technique using PDA against 10 fungi *viz. Alternaria* sp., *A. fumigatus*, *A.nidulans, A. niger*, *A. terreus*, *Bipolaris* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp. and *Penicillium* sp. isolated from cumin seed samples during mycological analysis [6].

Statistical analysis

The data were presented as mean ± standard error (SE), and each experiment was carried out in triplicate. SPSS software was used to conduct the statistical analysis (SPSS 16.0; IBM, NY, USA). The one-way analysis of variance (ANOVA) and Tukey's post-hoc test were used to assess treatment differences. P-values below 0.05 were regarded as statistically significant.

Results and Discussion

PH and Moisture content

The collected cumin seed samples showed variation in their pH and moisture content, indicating variability in storage duration and environmental conditions. The pH of cumin samples ranged from 5.63±0.10 to 5.76±0.16 (Table 1), showing a slightly acidic nature favorable for fungal colonization. Moisture content of the samples ranged from 13.64±1.00% in sample 3 to 14.23±0.55% in sample 1 (Table 1), indicating sufficient moisture to support fungal growth under prolonged storage. In stored cumin seeds, especially under conducive conditions, a slightly acidic pH and increased moisture content provide ideal conditions for fungal growth and mycotoxin synthesis [17]. More than 8–10% moisture content encourages the growth of storage fungus, including *Aspergillus, Penicillium, Fusarium* and others [18]. By increasing fungal enzyme activity and decreasing defense activity, these storage fungi thrive at slightly lower pH levels which promote colonization [19]. High temperature and humidity enhance respiration and lipid peroxidation, which further degrades seed quality and promotes the production of mycotoxin by *Aspergillus* species [20]. Therefore, to prevent fungal and mycotoxin contamination in cumin seeds when they are being stored under tropical circumstances, it is essential to maintain an ideal moisture content (less than 8%) and a neutral pH.

Table 1: Ph And Moisture Content (%) Of Collected Cumin Seed Samples

Cumin seed samples	pH	Moisture content (%)
Sample 1	5.60 ± 0.058^{a}	13.33 ± 0.118^{a}
Sample 2	5.31 ± 0.019^{a}	13.47 ± 0.019^{a}
Sample 3	5.51 ± 0.032^{a}	14.38 ± 0.032^{b}

Values are mean (n = 3) ± SE; $P < 0.05$. The means followed by same letter in the same column are not significantly different according to One-Way ANOVA and Tukey's multiple comparison tests

Mycological analysis of cumin seed samples

Mycological examination of the collected cumin seed samples revealed the occurrence of diverse fungal flora. Sample 3 showed the lowest occurrence frequency (30.91%) with a total of 311 isolates while highest (35.59%) in sample 2 with 358 isolates (Table 2). The fluctuation in frequency of occurrence across samples indicates that fungal colonization was influenced by variations in storage conditions, including temperature, moisture, and aeration [21]. A total of 11 identified fungal species belonging to seven genera were consistently isolated on Potato Dextrose Agar (PDA). The mycological analysis reflects the susceptibility of cumin seeds to colonization by a wide range of storage fungi, particularly under suboptimal storage environments. The sample 1 exhibited the highest relative density of *Cladosporium* sp (28.49%) followed by *Aspergillus niger* (24.33%) and *A. flavus* (16.91%) among 337 isolates. Sample 2 showed a total of 358 isolates in which *A. niger* showed highest relative density (35.20%) followed by *A. flavus* (20.11%) and *Cladosporium* sp. (14.80%) whereas, sample 3 showed highest relative density in *A. flavus* (27.01%) next to *Cladosporium* sp. (18.33%), *A. niger* (13.18%), *A. fumigates* (12.54%) and *Penicillium* sp. (10.61%) (Table 2, Figure 1). Collectively *Cladosporium* sp. exhibited highest relative density (21.86%) followed by *A. niger* (17.80%) and *A. flavus* (13.14%) (Table 2, Figure 2). The predominance of *Aspergillus* sp. and *Cladosporium* sp. are consistent with its role as a common airborne and surface contaminant thriving under high humidity [22]. Such dominancy indicates their adaptability prevailing during storage in hot and humid conditions [19]. A total 4.17% fungal isolates recovered during study were unidentified (Table 2) and point to potential novel or less-characterized fungal species that may require molecular identification for confirmation.

Table 2: Mycobiota analysis of collected cumin seed samples

Isolated Fungi	Cumin Sample 1	Cumin Sample 2	Cumin Sample 3	Total isolates	Relative density (%)
Alternaria sp.	6	2	7	15	2.29
Aspergillus candidus	3	0	6	9	1.37
Aspergillus flavus	28	37	21	86	13.14
Aspergillus fumigatus	13	10	18	41	6.26
Aspergillus niger	34	40	43	117	17.80
Aspergillus terreus	4	7	3	14	2.14
Aspergillus nidulans	2	0	5	7	1.07
Bipolaris sp.	9	14	16	39	5.96
Cladosporium sp.	38	47	58	143	21.86
Culvularia sp.	9	12	10	31	4.74
Fusarium sp.	12	17	19	48	7.33
Penicillium sp.	16	9	13	38	5.81
Unidentified	23	26	17	66	10.09
Total isolates	197	221	236	654	
Occurrence frequency (%)	30.12	33.79	36.08		

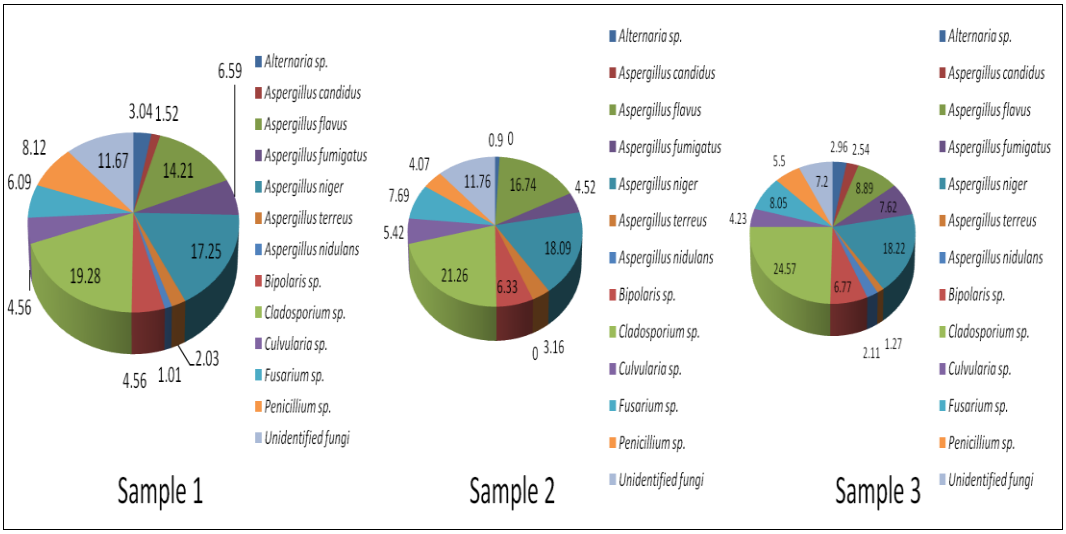

Fig 1: Relative densities (%) of isolated fungal species from cumin seed samples.

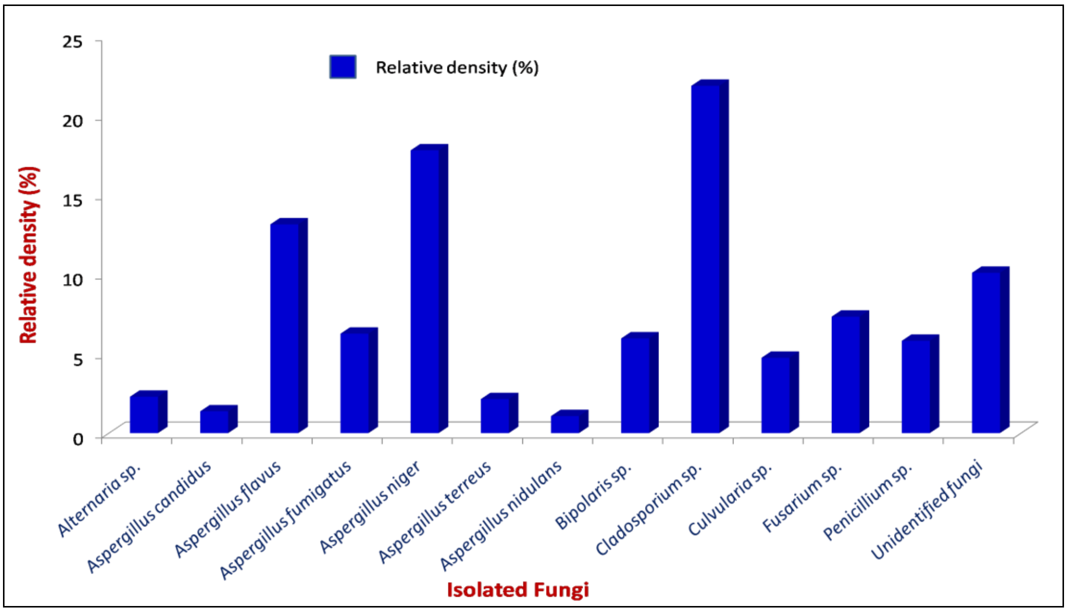

Fig 2: Cumulative relative densities of isolated fungal species from cumin seed samples.

Aflatoxigenic potential of isolated Aspergillus flavus

Ten isolates of *A. flavus* were randomly selected for aflatoxin production from individual cumin seed sample using TLC method revealed a significant toxigenic potential among the fungal populations associated with stored cumin seeds. Out of 10 isolates, 4 (40%) exhibited toxigenic potential. The highest aflatoxin B_1 production (1036.183µgL^{-1}) was reported from isolate *A. flavus* DDUCC-8 (Table 3) highlights its superior toxigenic capacity and selected as test fungus for further study. The predominance of *A. flavus* as both a frequent and toxigenic species emphasizes its ecological suitability and preference for substrates of cumin seeds, particularly under hot and humid storage conditions conducive to aflatoxin biosynthesis [19].

Table 3: Toxigenicity of *Aspergillus flavus* isolated from cumin seed samples.

Fungal isolates	AFB$_1$ (µgL^{-1})
A. flavus DDUCC-1	320.587
A. flavus DDUCC-2	-
A. flavus DDUCC-3	-
A. flavus DDUCC-4	749.945
A. flavus DDUCC-5	-
A. flavus DDUCC-6	-
A. flavus DDUCC-7	-
A. flavus* DDUCC-8	**1036.183**
A. flavus DDUCC-9	555.303
A. flavus DDUCC-10	-

* Fungal isolate ***A. flavus* DDUCC-8** from cumin seeds exhibited the highest aflatoxin B_1 producing potential

Extraction and composition of Cymbopogon citratus leaf essential oil (CCEO)

Essential oil extracted from *C. citratus leaf* via hydro-distillation yielded 1.8±0.4% (v/w) of very light greenish-yellow aromatic oil with lemony/citrusy scent. Gas Chromatography–Mass Spectrometry (GC–MS) profiling of CCEO revealed the presence of 23 phytochemical constituents, accounting for 94.16% of the total composition. E-Citral (60.98%) was found as major component followed by α-Sinensal (12.26%), Linalool (3.44%), Geraniol formate (2.59%) etc. while, rest other constituents were found in smaller quantities or in traces (Table 4). The predominance of E-Citral aligns with previous reports describing it as the major monoterpene hydrocarbon responsible for the characteristic citrus aroma and potent antioxidant, antimicrobial, and antifungal activities of CCEO [23, 24]. The presence of other significant constituents such as linalool, geraniol etc. further contributes to the oil's bioactivity and fragrance profile [25, 26]. The dominance of oxygenated monoterpenes and terpenoids suggests that the oil may possess significant biological potential, particularly as a natural preservative or antifungal agent [27, 28].

Table 4: Chemical profile of CCEO

S.No.	Retention time (min.)	Compounds	Concentration (%)
1.	8.133	α-Pinene	0.02
2.	11.125	6-Methyl-5-heptane-2-one	0.10
3.	12.931	S- (-)-Limonene	0.74
4.	14.800	Linalool oxide	0.08

5.	15.475	(Z)-Linalool oxide	0.06
6.	15.935	Linalool	3.44
7.	21.926	Nerol	0.40
8.	22.771	Z-Citral	2.19
9.	23.431	E-Citral	60.98
10.	24.001	2-Methyl-6-methylene-oct- 3,7-dien-2-ol	3.95
11.	25.381	Geraniol formate	2.59
12.	26.101	3-Hexxene-1-ol	0.16
13.	26.951	Caryophyllene oxide	0.18
14.	29.151	α-Sinensal	12.26
15.	29.751	β-Elemene	0.45
16.	35.251	2,3-Epoxy geranial	1.57
17.	36.576	Myrcenol	0.23
18.	37.026	Nerolidol	0.65
19.	38.251	β-Costol	0.08
20.	43.401	β-Farnesene	0.66
21.	44.501	Butanoic acid,3,7-dimethyl- 2,6-octadienyl ester	0.84
22.	50.251	β-Myrcene	0.19
23.	63.281	Trans-Geraniol	2.34
		Total	94.16%

Antifungal and antiaflatoxigenic activity of CLEO

CCEO exhibited potent fungitoxicity against *A. flavus* DDUCC-8 and its minimum inhibitory concentration (MIC) was recorded at 0.6 mgmL^{-1}. In addition, CCEO was also found efficient to inhibit the AFB_1 production and completely checked at 0.3 mgmL^{-1} (Table 5). A direct relation was found between fungal growth and AFB_1 production i.e. decreases in mycelial biomass resulted low AFB_1 production and vice versa. The observed inverse relationship between fungal biomass and AFB_1 production supports earlier findings that toxin biosynthesis is growth dependent and can be significantly reduced by disrupting cellular and metabolic processes [19]. One of the most widely recognized mechanisms is disruption of fungal cell membrane and cell wall integrity. Lipophilic EO components such as limonene, thymol, carvacrol, and citral penetrate the lipid bilayer, increasing membrane permeability, causing leakage of vital cellular contents (ions, proteins, nucleic acids), and leading to cell lysis [29, 30].

Table 5: Antifungal and antiaflatoxigenic activity of CLEO against *A. flavus* DDUAP3-7

Concentration (mgmL^{-1})	Biomass (g)	AFB_1 (µgL^{-1})
0	0.538±0.062^{c}	1432.114±126.019^{c}
2	0.128±0.018^{b}	279.786±54.364^{b}
4	0.048±0.010^{a}	67.326±18.253^{ab}
6	0.028±0.009^{a}	0.000±0.000^{a}
8	0.019±0.006^{a}	0.000±0.000^{a}
10	0.000±0.000^{a}	0.000±0.000^{a}

Values are mean (n = 3) ± SE; $P < 0.05$. The means followed by same letter in the same column are not significantly different according to One-Way ANOVA and Tukey's multiple comparison tests CCEO exhibited broad fungitoxic spectrum against some other storage fungi recovered from cumin seeds during mycological analysis. It completely checked the proliferation of all the tested fungal species at 0.6 mgml^{-1} (MIC against *A. flavus* DDUCC-8) except *Alternaria* sp. (89.96±3.00%), *Bipolaris* sp. (88.67±2.56%), *Cladosporium* sp. (72.31±2.22%) and *Curvularia lunata* (91.29±2.46%) (Table 6). The broad-spectrum fungitoxic activity of CCEO against various storage fungi suggests that its bioactive constituents, particularly E-citral and linalool, may interfere with membrane integrity and enzyme systems essential for fungal growth [31, 32].

Table 6: Fungitoxic spectrum of CCEO against other storage fungi

Fungal species	Percent inhibition at 0.6 mgmL^{-1}
Alternaria sp.	89.96±3.00^{b}
Aspergillus fumigatus	100.00±0.00^{c}
Aspergillus niger	100.00±0.00^{c}
Aspergillus terreus	100.00±0.00^{c}
Aspergillus nidulans	100.00±0.00^{c}
Bipolaris sp.	88.67±2.56^{b}
Cladosporium sp.	72.31±2.22^{a}
Curvularia sp.	91.29±2.46^{b}
Fusarium sp.	100.00±0.00^{c}
Penicillium sp.	100.00±0.00^{c}

Values are mean (n = 3) ± SE; $P < 0.05$. The means followed by same letter in the same column are not significantly different according to One-Way ANOVA and Tukey's multiple comparison tests

Conclusion

The results of this study offer a solid foundation for using CCEO as a multipurpose, natural preservative for spices and other goods that are kept in storage. Because to its high citral content, CCEO showed strong antifungal, antiaflatoxigenic, and antioxidant properties. It can also reduce losses from fungi and aflatoxin contamination in storage systems. The EO's promise as an ecofriendly phytopreservative for the safe storage of cumin seeds is highlighted by its broad-spectrum fungitoxicity and efficacy. In postharvest management systems, these results provide credence to the use of CCEO into botanical fungicide formulations as environment friendly substitutes for synthetic pesticides.

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