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# Assessment of Antifungal, Antiaflatoxigenic and Antioxidant Efficacy of *Ocimum Canum* Sims Essential Oil and E-Citral as Phytopreservative against *Aspergillus Flavus*, a Toxigenic Food Spoiling Mould

## Research Article

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**Abstract**

In the present study, stored wheat grains were found associated with spores of various storage moulds in which *Aspergillus flavus* exhibited the maximum relative density (33.48%). The chemical profile of *Ocimum canum* Essential oil (OcEO) showed 31 considerable peaks. E-citral (41.84%) was found as major component followed by Z-Citral (20.46%) and β-pinene (7.40%). OcEO and E-citral both exhibited broad spectrum fungitoxicity and their MIC against *A. flavus* LHPTA-07 was recorded at 0.6 and 0.4 mg/ml respectively while aflatoxin $B_1$ production was completely checked at 0.4 and 0.3 mg/ml respectively. The gradual reduction in ergosterol content with increasing OcEO and E-citral concentration indicates plasma membrane as the possible target site of antifungal action. OcEO and E-citral also exhibited significant antioxidant potential with $IC_{50}$ value (16.72 and 15.44 µg/ml respectively) comparable to synthetic antioxidants (ascorbic acid, $IC_{50}$ 9.76 µg/ml) would be helpful to minimize lipid peroxidation. Fumigation of OcEO and E-citral significantly reduced the population of *A. flavus* from wheat grains without adversely affecting seed germinability. Hence, OcEO and E-citral exhibited special virtues possessing antifungal, antiaflatoxigenic and antioxidant activity as well as no adverse effects on seed viability strengthening their safe exploitation as green preservative to enhance the shelf life of stored food commodities and other edible products.

**Keywords:** *Aspergillus flavus*; *Ocimum canum*; Essential oil; Antifungal; Aflatoxin; Antioxidant

## Introduction

Food commodities are susceptible to fungal and mycotoxin contamination during postharvest storage and illness due to consumption of these contaminated food products is a priority concern to public health. In developing countries, postharvest economic losses reach 25-40% or even more by fungal contamination and the mycotoxins produced by them [1]. Among storage moulds, different species of *Aspergillus* potentially contaminate stored food commodities and adversely affect their nutritive values [2]. Toxigenic species of *Aspergillus* secrete aflatoxins in stored food commodities and majority of population in developing countries are exposed to aflatoxicoses [3]. Aflatoxin $B_1$ is potent hepatocarcinogenic and immunosuppressive, therefore, classified as group-1 human carcinogen by International Agency for Research on Cancer [4].


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Various synthetic fungicides (Carbendazim, Mancozeb, Benomyl, Ceresan, Ziram etc.) have been used for a long time and have greatly contributed in management of such losses but due to their harmful health effects, resistance development in pathogens and residual toxicity [5]. Synthetic preservatives are also produce partially reduced oxides such as superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$) and also hydroxyl radicals ($OH^-$) causing severe damage to biomolecules and also responsible for the stimulation of aflatoxin biosynthesis [6,7]. Hence, there is a need to develop some ecofriendly alternatives of these synthetic fungicides/preservatives.

Higher plants are great reservoir of various bioactive phytochemicals. Recently, plant based formulations are exploited as safe alternatives of synthetic preservatives and these green preservatives are Generally Recognized as Safe (GRAS) by U.S. FDA [8]. Various essential oils and their common bioactive components *viz.* terpenoids and phenolics have been used as antifungal [9], antimycotoxigenic [10], and pesticidal as well as potent free radical scavengers [11,12]. Some essential oil based formulations viz. TALENT, EcoSMART and EcoPCOR have been used on large scale in food and agriculture industries [13].

In the present study, *Ocimum canum* Essential oil (OcEO) and its major component E-citral has been investigated for their fungitoxicity against toxigenic isolate of *A. flavus*, a potent storage fungus causing postharvest loses of food commodities. In addition, OcEO and E-citral was also studied for their antioxidant activity as well as effect on seed viability for recommendation of OcEO as plant based food preservative.

## Materials and Methods

### Collection and preparation of wheat grains

Stored wheat (*Triticum aestivum* L.) grains (variety-Malviya) were locally procured from retailers of Varanasi district of Uttar Pradesh, India. The grains were collected in sterilized low density poly ethylene bags to avoid further contamination. The grains were ground using a surface sterilized household blender. The powder was filtered through No. 50 mesh sieve and packed tightly in paper bags and stored at 5±2 °C for further analysis [14].

### pH and moisture content determination

**For pH measurement, powdered wheat sample:** distilled water suspension (1:10; w/v) was prepared and stirred for 24 h in 200 ml beaker. The pH of the suspension was measured using electronic pH meter. To determine moisture content, weighed amount of powered samples were dried at 100 °C until their weights remained constant and per cent moisture content was calculated as follows [15] -

$$\% \text{ moisture content} = \frac{\text{Undried sample wt.} - \text{Dried sample wt.}}{\text{Undried sample wt.}} \times 100$$

### Mycological analysis of powdered wheat samples

Storage moulds associated with wheat grains were assessed by serial dilution method [16]. The isolated fungal species were identified on the basis of cultural and morphological characteristics [17,18]. The identified fungal colonies were purified and preserved on Potato Dextrose Agar (PDA) slant at 4±2 °C. The per cent relative densities of different fungi on raw herbal drug samples were calculated following [19].

### Detection of aflatoxigenic isolate of *A. flavus*

Twenty isolates of *A. flavus* were randomly selected to determine their Aflatoxin $B_1$ producing potential by Thin Layer Chromatography (TLC) following [20]. Fifty µl conidial suspension (≈$10^6$ conidia/ml) of selected *A. flavus* isolates were separately inoculated in 49.5 ml SMKY (Sucrose, 200 g; $MgSO_4.7H_2O$, 0.5 g; $KNO_3$, 0.3 g; Yeast extract, 7.0 g; Distilled water, 1000 ml) broth medium in 150 ml Erlenmeyer flask and mixed properly followed by incubation at 27±2 °C for 10 days. Content of each flask was filtered after incubation and filtrate was extracted with chloroform (40 ml) in a separating funnel. The separated chloroform extract was dried on water bath at 60-70 °C. The residue left after evaporation was re-dissolved in 1 ml chloroform and 50 µl of it was spotted on TLC plate (20×20 $cm^2$ of silica gel). The plate was developed in toluene: isoamyl alcohol: methanol (90:32:2; v/v/v) solvent system and intensity of $AFB_1$ was observed under ultra violate fluorescence analysis cabinet at an excitation wavelength of 360 nm [21]. The fluorescent blue spots on TLC plate containing $AFB_1$ were scraped in 5 ml cold methanol and centrifuged at 3000 rpm for 5 min. Absorbance of supernatant was recorded at 360 nm and $AFB_1$ content was quantified following [22].

$$AFB_1 \text{ content } (\mu g/L) = \frac{D \times M}{E \times L} \times 1000$$

Where, D = absorbance, M = molecular weight of $AFB_1$ (312), E = molar extinction coefficient of $AFB_1$ (21,800) and L = path length (1 cm cell was used)

### Extraction of *O. canum* leaf essential oil (OcEO)

For essential oil extraction, leaves of *O. canum* were collected from Banaras Hindu University campus, Varanasi. The plant was identified with the help of Flora of BHU Campus and its voucher specimen (Lam/O-119/2019) was lodged in the herbarium of department of botany [23], Banaras Hindu University. Leaves of the plant were thoroughly washed with 1% Sodium hypochlorite followed by distilled water. The volatile fraction (EO) of leaves was extracted by Clevenger's hydro-distillation apparatus. The extracted OcEO was stored in dark clean glass vial after removing water traces passing through anhydrous sodium sulphate and kept at 4-6 °C [21].

### GC/GC-MS analysis of OcEO

To determine the chemical composition, OcEO was analyzed through gas chromatography (Perkin Elmer Auto XL GC) equipped with a flame ionization detector. The GC conditions were as follows: column, EQUITY-5 (60 m x 0.32 mm x 0.25 µm); $H_2$ was the carrier gas; column Head pressure 10 psi; oven temperature program isotherm 2 min. at 70 °C, 3 °C/min. gradient to 250 °C, isotherm 10 min; injection temperature, 250 °C; detector temperature 280 °C. The GC-MS analysis was also performed using Perkin Elmer Turbomass GC-MS. The effluent of the GC column was introduced directly into the source of MS. Spectra were obtained in the EI mode with 70ev ionization energy. The compounds were identified by comparison of their relative retention times and the mass spectra with those of authentic reference compounds shown in the literature [24].


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### Fungitoxic spectrum of OcEO and E-citral against some storage fungi

Fungitoxic efficacy of OcEO and its major component E-citral was also recorded against 12 storage moulds *viz. Alternaria alternata, Aspergillus candidus, A. flavus, A. fumigatus, A. nidulans, A. niger, A. terreus, Cladosporium cladosporioides, Curvularia lunata, F. oxysporum, Penicillium italicum and Trichoderma viride* recovered from powdered wheat samples. Requisite amount of OcEO and E-citral dissolved separately in 0.5 ml of 5% tween-20 mixed with 9.5 ml PDA medium in different presterilized Petri dishes to attain final concentrations i.e. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5 and 2.0 mg/ml. The control sets were kept parallel to the treatment sets without OcEO. A positive control of bavistin (Carbendazim 50% WP), a prevalent synthetic fungicide was also kept parallel. A fungal disc (5 mm diameter) of each test fungus was inoculated separately and incubated at 27±2 °C for 7 days. After incubation, Minimum Inhibitory Concentration (MIC) was recorded [25]. The concentrations on which OcEO and E-citral completely checked the visible fungal growth on PDA medium was considered as MIC.

### Antifungal and aflatoxin inhibitory efficacy of OcEO and E-citral

Fungitoxic and aflatoxin inhibitory efficacy of isolated OcEO and its major component E-citral was tested against the toxigenic isolate of *A. flavus* LHPTA-07 using SMKY (Sucrose, 200 g; $MgSO_4.7H_2O$, 0.5 g; $KNO_3$, 0.3 g; Yeast extract, 7.0 g; Distilled water, 1000 ml) broth as nutrient medium following [20]. Requisite amount of the OcEO and E-citral dissolved separately in 0.5 ml of 5% tween-20 were pipetted aseptically to different presterilised Erlenmeyer flasks (150 ml) containing 49.5 ml of SMKY broth to procure the final concentrations viz. 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg/ml. The control sets were kept parallel to the treatment sets without OcEO and E-citral. Then, flasks were inoculated aseptically with 50 µl spore suspension (≈$10^6$ spores $ml^{-1}$) of toxigenic isolate of *A. flavus* LHPTA-07 prepared in 0.1 % Tween-80 and incubated at 27±2 °C for 10 days [26]. The content of each flask was filtered (Whatman no. 1) and mycelium was oven dried at 100 °C till their weight remained constant for biomass determination. Mycelial biomass of treatment and control sets was measured and per cent mycelial inhibition was calculated [21]. The filtrates of control and treated sets were extracted separately with 50 ml chloroform in a separating funnel to quantify the aflatoxin $B_1$ production. Aflatoxin $B_1$ production in each set was estimated by aforementioned technique of [20].

### Effect of OcEO and E-citral on ergosterol content

Effect of OcEO on ergosterol content in plasma membrane of *A. flavus* LHPTA-07 was assessed following [22]. Requisite amount of the OcEO and E-citral was dissolved separately in 0.5 ml of 5% tween-20 were pipetted aseptically to different presterilised Erlenmeyer flasks (150 ml) containing 49.5 ml of SMKY broth to procure the final concentrations from 0.1 to 0.6 mg/ml. The flask without OcEO and E-citral was treated as control. Each flask was inoculated with 100 µl spore suspension of *A. flavus* LHPTA-07 followed by incubation at 27±2 °C for 5 days. Recovered mycelia from treated and control sets were subjected to extraction and quantification of ergosterol.

### Free radical scavenging activity of OcEO and E-citral

The antioxidant activity of the OcEO and E-citral was determined by DPPH radical scavenging assay on TLC as well as its free radical scavenging activity was measured through spectrophotometery following [27]. Free radical scavenging activity of the OcEO and E-citral was measured by recording the extent of bleaching of the purple-coloured DPPH solution to yellow. Different graded concentrations (1.0 to 20.00 µg/ml) of the samples were added separately to 4% DPPH solution in methanol (5 ml). After a 30 min of incubation at room temperature, the absorbance was taken against a blank at 517 nm using spectrophotometer. Scavenging of DPPH free radical with reduction in absorbance of the sample was taken as a measure of their antioxidant activity following [28]. Ascorbic acid was used as positive control. Per cent Free Radical Scavenging Activity (FRSA) was calculated using the following formula –

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

Where, $A_{blank}$ is the absorbance of the control (without test compound), and $A_{sample}$ is the absorbance of the test compound.

### Antifungal fumigant activity of OcEO and E-citral during storage

To determine the antifungal efficacy of OcEO during storage, 500 g of surface sterilized wheat grains were kept separately in four sets in different plastic containers having aerial volume 2.0 liters. Each set (three containers) was inoculated with 1 ml spore suspension (≈$10^6$ spores/ml) of *A. flavus* LHPTA-07. Each set was fumigated with OcEO (0.6 mg/ml), E-citral (0.4 mg/ml) and bavistin (0.8 mg/ml) separately at their MIC against *A. flavus* one set run parallel as control without any fumigation. All the containers were kept air tight and stored for six months at room temperature i.e. 27±2 °C [29,30]. After storage, mycological analysis of fumigated wheat grains was performed to determine the effect of fumigation on number of isolates of *A. flavus* following [16].

### Seed germination test

The viability of fumigated wheat grains was tested by seed germination test. After six months of storage, 200 uninfested seeds were taken from each treated group and soaked in distilled water for 3 h. Thereafter, seeds were aseptically transferred to Petri dishes of 15 cm diameter (20 seeds per Petri plate) containing moist filter paper and incubated at 25±2 °C. Two hundred healthy and uninfested seeds were taken from the market as control for comparison. The number of seeds germinated within a week was recorded as viable [31].

### Statistical analysis

All the experiments were accomplished in triplicate and data were expressed as Mean±Standard error (SE) followed by one way ANOVA ($P < 0.05$) and Tukey's multiple range tests. The software SPSS (version 16.0) was used for statistical analysis of data.

## Results and Discussion

### Mycoflora analysis of powdered wheat grains

The level of fungal contamination is mainly governed by pH and moisture content. The collected wheat grains having appropriate


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pH (6.90±0.047) whereas their relatively higher moisture content (17.64±0.347%) reflects effect of conducive climatic conditions of Indian subcontinent which enhance fungal and mycotoxin contamination in food commodities during storage. During mycological analysis, total 1335 fungal isolates were recovered and among them A (Figure 1). flavus was dominated with highest relative density (33.48%) followed by *Cladosporium cladosporioides* (20.92%) and *A. niger* (17.83%) while *A. nidulans* exhibited the lowest (1.05%) relative density (Figure 2). Thirty percent isolates of randomly selected *A. flavus* were found toxigenic and *A. flavus* LHPTA-07 was selected as test fungus due to its higher potential of afltoxin $B_1$ production (2392.654 µg/l) presented in Table 1. The Aspergilli usually exhibited relatively higher density due to their strong capability to produce some hydrolytic enzymes [32-34]. Fungal contamination also degraded the nutritive values of food commodities and rendering them unfit for human consumption [2]. Some higher plant essential oils can be exploited as substitute of synthetic fungitoxicant against several moulds and their mycotoxin production [10,35]. An effort was made to evaluate the antifungal efficacy of OcEO and its possible applicability in control of postharvest fungal deterioration of food commodities during storage and also enhancing their shelf life.

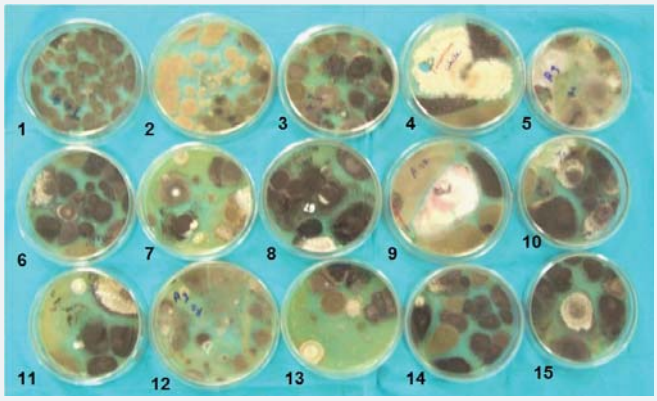


**Figure 1:** Fungal species recovered from powdered wheat grains by serial dilution technique.

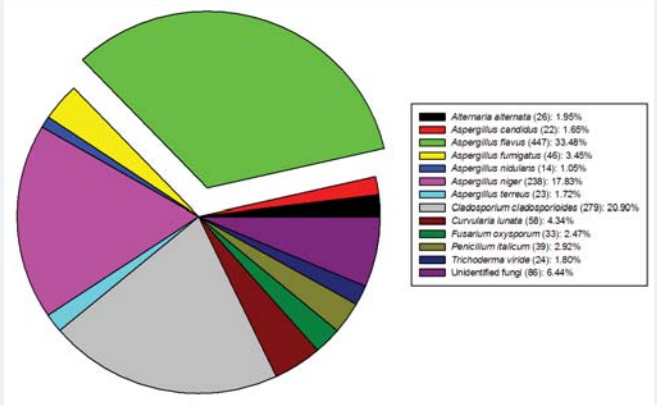


**Figure 2:** Relative densities (%) of recovered fungi from powdered wheat grains by serial dilution technique.

**Table 1:** Detection of aflatoxigenic potential of *A. flavus* isolates.

| Fungal isolates | Toxigenicity | $AFB_1$ content (µg/l) |
|---|---|---|
| *A. flavus* LHPTA-01 | Non-toxigenic | - |
| *A. flavus* LHPTA-02 | Non-toxigenic | - |
| *A. flavus* LHPTA-03 | Non-toxigenic | - |
| *A. flavus* LHPTA-04 | Toxigenic | 835.816 |
| *A. flavus* LHPTA-05 | Non-toxigenic | - |
| *A. flavus* LHPTA-06 | Non-toxigenic | - |
| ***A. flavus* LHPTA-07** | **Toxigenic** | **2392.954** |
| *A. flavus* LHPTA-08 | Non-toxigenic | - |
| *A. flavus* LHPTA-09 | Non-toxigenic | - |
| *A. flavus* LHPTA-10 | Toxigenic | 1637.284 |
| *A. flavus* LHPTA-11 | Non-toxigenic | - |
| *A. flavus* LHPTA-12 | Non-toxigenic | - |
| *A. flavus* LHPTA-13 | Non-toxigenic | - |
| *A. flavus* LHPTA-14 | Non-toxigenic | - |
| *A. flavus* LHPTA-15 | Toxigenic | 1797.578 |
| *A. flavus* LHPTA-16 | Toxigenic | 1499.900 |
| *A. flavus* LHPTA-17 | Non-toxigenic | - |
| *A. flavus* LHPTA-18 | Toxigenic | 1076.257 |
| *A. flavus* LHPTA-19 | Non-toxigenic | - |
| *A. flavus* LHPTA-20 | Non-toxigenic | - |

*The fungal isolates in bold is detected as most toxigenic

## Extraction and chemical description of OcEO

The OcEO was extracted through hydro-distillation is characterized with its pungent smell, yellow green colour and 0.96 % yield (w/w). The chemical profile of EOs varies with age of the plant, season of collection, geographical area and soil characteristics [36,37]. Hence, OcEO was standardized to determine the chemical composition through GC-MS analysis before recommending for formulation. The GC-MS analysis of OcEO showed 31 considerable peaks in which E-Citral (41.84%) was found as major component followed by Z-Citral (20.46%), β-Pinene (7.40%), Caryophyllene oxide (5.04%), Epoxy ocimene (3.54%), Trans Carvone oxide (3.12%), β-Costol (2.67%) and 3-Heptadecen-5-yne (2.03%). Rest other identified components were found in trace amount (Table 2). The major component of OcEO is different from earlier findings where, camphor [38], linalool [39], eucalyptol [40], thymol [41], etc. were reported as major components.

## Antifungal and antiaflatoxigenic efficacy of OcEO and E-citral

The OcEO and E-citral both exhibited remarkable broad fungitoxic spectrum at 1.0 mg/ml concentration against the 12 storage fungi isolated during mycological analysis and also showed superiority over synthetic fungicide bavistin (Figure 3). The broad spectrum fungitoxicity and superiority over bavistin would be suitable to provide complete protection from large number of fungal pathogens. The MIC of OcEO and E-citral against *A. flavus* LHPTA-07 was found to be 0.6 mg/ml and 0.4 mg/ml respectively whereas; OcEO and E-citral completely checked the $AFB_1$ production at 0.4 and 0.3 mg/ml concentration respectively (Table 3 and Figure 4). A direct relationship has been observed between fungal biomass and aflatoxin production. The $AFB_1$ production decreased with


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**Table 2:** Chemical profile of OcEO by GC/GC-MS analysis.

| RT | Compounds | Percentage |
|---|---|---|
| 7.201 | 3-Methyl-2-heptanone | 0.28 |
| 8.401 | 2-Methylcyclopentanol acetate | 0.31 |
| 10.476 | 2,6-Dimethyl-2,7-octadiene-1,6-diol | 0.23 |
| 11.177 | 6-Methyl-5-hepten-2-one | 0.17 |
| 11.301 | 4-Methyl-1-buten-1-yl ester pentanoic acid | 0.18 |
| 12.951 | Linalool oxide | 0.49 |
| 14.826 | Caryophyllene diepoxide | 1.38 |
| 15.501 | Linalol oxide | 0.46 |
| 15.901 | β-Pinene | 7.40 |
| 19.226 | Epoxy linalol | 0.21 |
| 19.401 | 2-Nonyne | 0.14 |
| 20.351 | D-Nerolidol | 0.46 |
| 21.751 | 4-Methyl-4-hepten-3-ol | 0.62 |
| 21.926 | Linalyl propionate | 0.50 |
| 22.551 | **Z-Citral** | **20.46** |
| 23.126 | Linalyl acetate | 0.40 |
| 23.301 | 3-Methyl-6-(1-methylethyl)-2-cyclohexen-1-one | 0.27 |
| 23.951 | **E-Citral** | **41.84** |
| 24.451 | Epoxy ocimene | 3.54 |
| 24.676 | Trans carvone oxide | 3.12 |
| 25.376 | Tetrahydroionone | 1.72 |
| 26.526 | Lancifolol | 0.12 |
| 27.101 | Caryophyllene oxide | 5.04 |
| 27.926 | Neric acid | 0.22 |
| 28.776 | 3-Heptadecen-5-yne | 2.03 |
| 30.326 | Chrysanthenone | 1.12 |
| 31.401 | 2,3,4,5-Tetramethyl cyclopent-2-en-1-ol | 1.03 |
| 38.276 | β-Costal | 2.67 |
| 58.202 | Citronellyl acetate | 0.48 |
| 60.652 | α-Farnesene | 0.72 |
| 61.727 | 3,7-Dimethyl-2,6-octadienal | 0.58 |

RT: Retention Time; Compounds in bold are major components

increasing concentrations of OcEO and E-citral. Fungal biomass and $AFB_1$ production exhibited a significant declining trend with increasing OcEO and E-citral concentrations. Therefore, to check $AFB_1$ production, mycelial growth must be below the threshold limit so that aflatoxin could not be produced [42]. The OcEO completely checked the $AFB_1$ production at lower concentration than earlier reported EOs *viz*. *Cymbopogon flexuosus* [31], *Curcuma longa* [43], *Pimenta dioica* [44], *Coriandrum sativum* [45], *Artemisia nilagirica* [46] etc. The antifungal and antiaflatoxigenic efficacy of OcEO may be due to bioactivity of different constituents or due to different metabolic pathways because; MIC and $AFB_1$ inhibition was recorded at different concentrations. E-citral, a well known antifungal agent [47,48] is major component (41.84%) of OcEO, may also played promising role in its fungitoxicity.

## Antioxidant activity of OcEO and E-citral

Food commodities are also deteriorated by free radical mediated oxidation of unsaturated lipids during storage [49]. Oxidative stress stimulates *A. flavus* to produce more $AFB_1$ during storage [7] which results quantitative as well as qualitative losses to stored commodities and reduces their shelf life. The OcEO and E-citral exhibited significant radical scavenging activity as their $IC_{50}$ values (16.72 and 15.44 µg/ml respectively) in concentration dependent manner (Figure 5), which were found comparatively higher than ascorbic acid (9.76 µg/ml). The $IC_{50}$ values of OcEO and E-citral were found greater than EO of *O. sanctum*, *O. gratissimum*, *O. basilicum* etc. but quite lower than some earlier reported EOs and also comparable to synthetic antioxidants [39,50,51]. The presence of various phenolic compounds and/or synergistic effect among compounds also play major role in antioxidant activity of EOs [52,53]. Owing to free radical scavenging activity, the OcEO and E-citral may serve as plant based antioxidants in shelf life enhancement as well as protection from oxidative stress by decelerating oxidative rancidity of lipids.

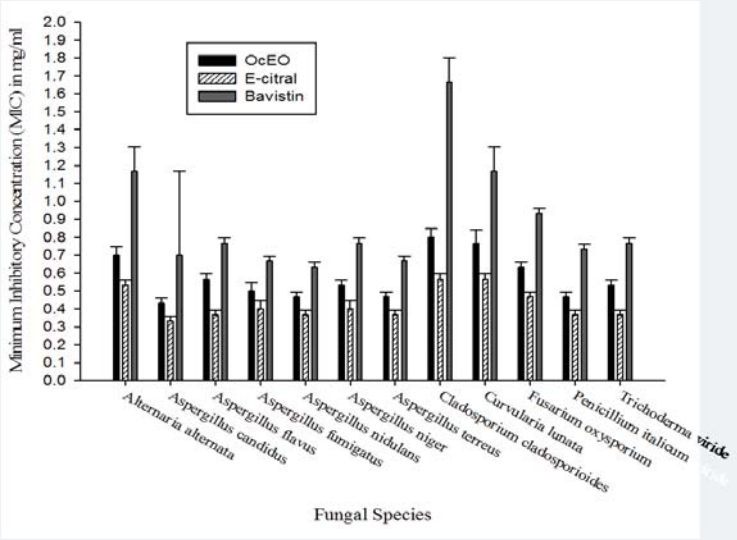


**Figure 3:** Fungitoxic spectrum of OcEO and E-citral against some storage moulds and its comparison with Bavistin.



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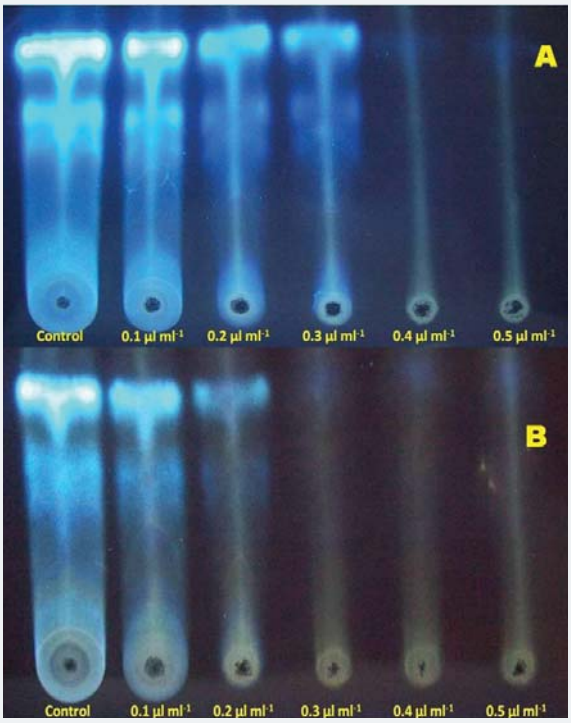


**Figure 4:** Fluorescent TLC plate showing antiaflatoxigenic efficacy - (A) OcEO and (B) E-citral.

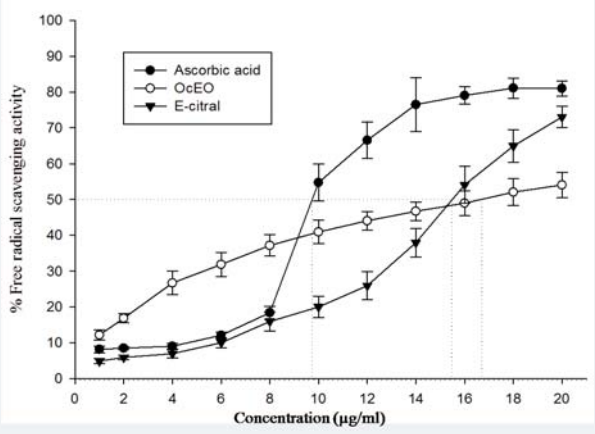


**Figure 5:** Free radical scavenging activity of OcEO and E-citral.

### Effect of OcEO and E-citral on fungal cell membrane

Ergosterol is specific sterol in fungal cell membrane providing membrane integrity and flexibility as well as stability of membrane associated enzymes and significant alteration of its biosynthesis adversely affect fungal growth [54,55]. Ergosterol content of cell membrane was found decreasing with increasing concentration of OcEO and E-citral. The per cent inhibition of ergosterol contents by OcEO treatment at 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/ml concentration was 33.86%, 55.02%, 77.14%, 86.43%, 92.86% and 100% respectively. Whereas per cent ergosterol content inhibition by E-citral at 0.1, 0.2, 0.3 and 0.4 mg/ml concentration was found 43.57%, 65.71%, 85.71% and 100% respectively (Figure 6). The decrease in ergosterol level with increasing OcEO and E-citral concentration clearly denotes that the bioactive components of OcEO targeted the cell membrane and rendering them more permeable to leakage of ion and other cell content [56].

### Effect of OcEO and E-citral on seed germinability

The OcEO and E-citral drastically reduces the *A. flavus* population in fumigated wheat samples i.e. 75.19 and 80.73% at 0.6 mg/ml and 0.4 mg/ml concentrations respectively (Table 4). The fumigant activity of OcEO and E-citral against *A. flavus* was high and also comparable to vabistine where 73.95% reduction was recorded (Table 4). Reduction of *A. flavus* isolates in fumigated wheat samples of OcEO and its major constituent also showed their preservative nature. OcEO and E-citral fumigated wheat grains showed 81.5% and 78.0% germination, which was also noteworthy (86.5%) to prevalent synthetic fungicide vabistine strengthens their non-phytotoxic nature even six months after application (Table 5).

The plant *O. canum* is also used as phytomedicine in different diseases [57,58] exhibited its non-mammalian toxic nature. Thus, non-phytotoxic and non-mammalian toxic nature of the OcEO strengthens its possible exploitation as a safer plant based preservative of food commodities during storage. The findings may draw the attention of food industries to conduct further experiments regarding large scale exploitation of OcEO as botanical preservative for food commodities during storage. The attraction of modern society in 'green consumarism' [59] desiring fewer synthetic ingredients in foods and recommendation of herbal products as 'Generally Recognized as Safe' (GRAS) in the developed countries may lead scientific interest in OcEO as food preservative.

**Table 3:** Effect of OcEO and E-citral concentrations on *A. flavus* LHPTA-07 biomass and $AFB_1$ production.

| Concentration (mg/ml) | OcEO | | E-Citral | |
|---|---|---|---|---|
| | Fungal Biomass (g) | $AFB_1$ production (µg/l) | Fungal Biomass (g) | $AFB_1$ production (µg/l) |
| Control | 0.402 ± 0.028$^{a}$ | 2282.275 ± 110.265$^{a}$ | 0.402 ± 0.028$^{a}$ | 2282.275 ± 110.265$^{a}$ |
| 0.1 | 0.239 ± 0.030$^{b}$ | 854.899 ± 87.921$^{b}$ | 0.168 ± 0.013$^{b}$ | 412.177 ± 47.980$^{b}$ |
| 0.2 | 0.132 ± 0.009$^{c}$ | 263.339 ± 32.385$^{c}$ | 0.069 ± 0.006$^{c}$ | 93.505 ± 10.907$^{c}$ |
| 0.3 | 0.079 ± 0.006$^{d}$ | 89.679 ± 17.559$^{d}$ | 0.023 ± 0.002$^{d}$ | 0 |
| 0.4 | 0.036 ± 0.005$^{de}$ | 0 | 0 | - |
| 0.5 | 0.013 ± 0.002$^{e}$ | 0 | - | - |
| 0.6 | 0 | - | - | - |

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



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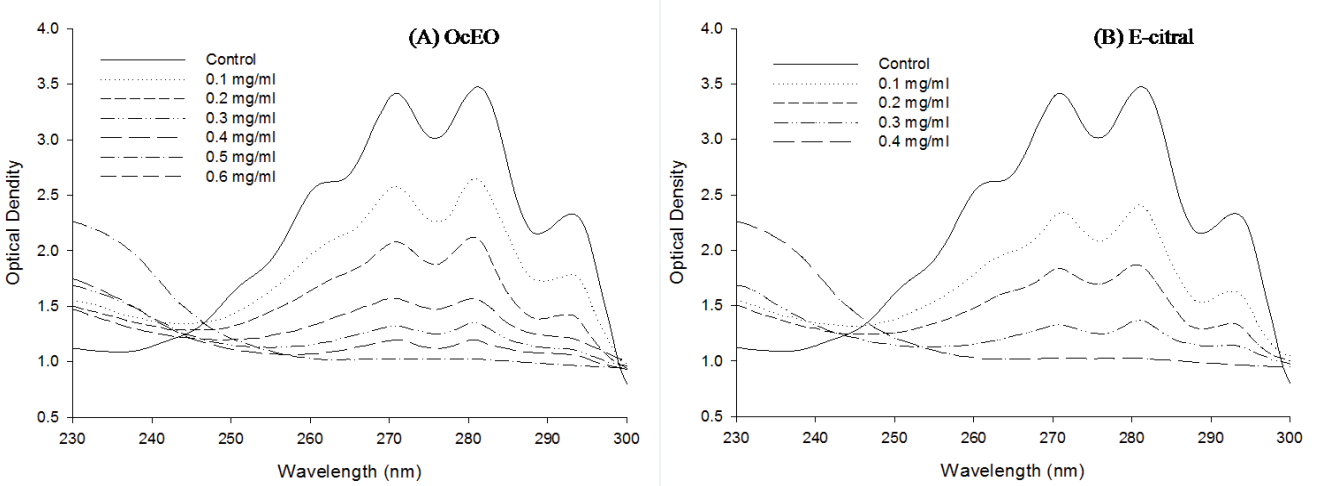


**Figure 6:** Effect of OcEO and E-citral concentration of ergosterol content on fungal cell membrane.

**Table 4:** Per cent inhibition in A. flavus isolates after six months fumigation.

| Treatment (mg/ml)* | No. of *A. flavus* isolates | Per cent inhibition |
|---|---|---|
| **Control** | **1282** | **-** |
| OcEO (0.6 mg/ml) | 318 | 75.19 |
| E-citral (0.4 mg/ml) | 247 | 80.73 |
| Bavistin (0.8 mg/ml) | 334 | 73.95 |

*The wheat samples were fumigated at MIC by individual treating agent

**Table 5:** The effect of OcEO, E-citral and bavistin treatment on germinability of wheat grains.

| Treatment (mg/ml) | % Germination |
|---|---|
| Control | 88.50 ± 7.47[a] |
| OcEO (0.6 mg/ml) | 81.50 ± 6.26[b] |
| E-Citral (0.4 mg/ml) | 78.00 ± 5.37[c] |
| Bavistin (0.8 mg/ml) | 86.50 ± 5.50[a] |

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

## Conclusion

The findings of present study reveals that, OcEO exhibited antifungal, antiaflatoxigenicity, broad fungitoxic spectrum, free radical scavenging activity and non-phytotoxicity which strengthening its exploitation as a substitute of synthetic preservatives for enhancing the shelf life of stored food commodities and other edible products during storage.

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