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In vitro assessment of *Ocimum basilicum* essential oil as phytopreservative against fungal contamination of groundnut seeds during storage

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Abstract

Groundnut (*Arachis hypogaea L.*) is an important oilseed crop rich in edible oil, proteins, vitamins, and minerals, contributing significantly to human nutrition as well as improves soil fertility through nitrogen fixation. In the present investigation, pH and moisture content of collected groundnut seeds was 6.71 and 23.82% respectively. Groundnut seeds were found highly prone to fungal infestation during storage. Mycological examination of stored groundnut seeds revealed a total of 1194 isolates from six different known genera and also including some unidentified forms. Aspergilli were predominance including *Aspergillus flavus*, *A. fumigates*, *A. Niger*, and *A. terreus*. The highest relative density was recorded in *A. flavus* (25.29%) followed by *Penicillium* spp. (18.26%) and *A. Niger* (13.14%), whereas the lowest relative density was found in *Alternaria* spp. (3.01%) next to unidentified fungi (3.52%). The *Ocimum basilicum* essential oil (OBEO) exhibited potent antifungal activity against *A. flavus* and its minimum inhibitory concentration (MIC) was recorded at 2.0 µl/ml. The OBEO also exhibited broad fungitoxic spectrum against 8 storage fungi recovered from groundnut seeds. The study highlights the potential of OBEO as an eco-friendly and effective phytopreservative for improving the storage quality and safety of groundnut seeds.

Keywords: *Ocimum basilicum*, essential oil, *groundnut, Aspergillus flavus,* antifungal, phytopreservative

Introduction

Groundnut (*Arachis hypogaea* L.) is a globally important oilseed crop valued for its nutritional and economic significance. However, during post-harvest storage, groundnut seeds are highly prone to fungal infestation, particularly by *Aspergillus* species, which not only reduce seed quality but also produce aflatoxins [1, 2]. These mycotoxins pose serious health hazards, including hepatotoxic, immunosuppressive, and carcinogenic effects, thereby threatening food safety and trade [3]. Conventional control of storage fungi largely depends on synthetic fungicides and chemical preservatives, the excessive use of which raises concerns related to environmental pollution, chemical residues, fungal resistance, and risks to human health [4].

In recent years, plant-based essential oils have emerged as promising eco-friendly alternatives for managing storage fungi and mycotoxin contamination. Essential oils are rich in biologically active compounds such as phenolics and terpenoids that exhibit strong antifungal and antiaflatoxigenic properties [5]. *Ocimum basilicum* L. (sweet basil) is a widely cultivated aromatic herb whose essential oil is known for its antimicrobial, antioxidant, and preservative potential, attributed to constituents like linalool, eugenol, methyl eugenol, and cineole [6, 7].

The present study investigates the *in vitro* efficacy of *O. basilicum* essential oil as a phytopreservative against fungal growth in stored groundnut seeds. The findings aim to support its potential use as a natural, safe, and sustainable alternative to chemical preservatives for post-harvest protection.

Materials and Methods

1. Collection of groundnut samples

Stored groundnut seeds sample was procured from the local retailer of Maharajganj district, Uttar Pradesh, India, in October, 2025. The stored groundnut sample (500 g) was collected in sterile polythene bags (20×16 cm^2) to avoid further contamination and stored in laboratory at 4±2°C [8].

2. Determination of pH and Moisture content (%)

The collected groundnut seeds was finely ground using a sterile grinder. Ten grams of powdered groundnut seeds were suspended in 100 mL distilled water (1:10 w/v ratio), shacked well for 30 minutes on rotary shaker to ensure uniform extraction of soluble components, and then allowed to settle. The pH of the supernatant is measured using a calibrated digital pH meter [9].

To determine the moisture content (%)**,** 50 g of collected groundnut sample was weighed and oven dried at 100±2°C until their weight remains constant [10]. The moisture content (%) was calculated as follows:

Moisture content (%) = $(I_W - F_W / I_W) \times 100$

Where I_W is the initial weight and F_W is the final weight after drying.

3. Mycoflora analysis of groundnut seed sample:

One gram of powdered groundnut seeds was suspended in 9.0 ml sterile 0.85 % saline solution in the test tube and was thoroughly homogenized using vortexer for 5 minutes. Three folds of serial dilution were prepared following [11]. The sample (0.5 ml) of appropriate dilution (10^{-3}) was pipetted onto sterile Petri plates containing Potato Dextrose

Agar (Potato, 200g; Dextrose, 20g; Agar, 18g and distilled water 1000 ml) medium supplemented with streptomycin to suppress bacterial growth. The inoculum was evenly spreaded using a sterile glass spreader. Inoculated plates were incubated at 27±2°C for 7 days and examined daily but counts were recorded only after 3-4 days. Every mold colony with a unique morphology was recognized after being subcultured on PDA [12, 13]. The relative density of each fungal isolate was calculated in accordance with the method proposed by Kumar *et al*. [14].

$$\text{Relative Density of fungus (\%)} = \frac{\text{No. of isolates of a fungus}}{\text{Total no. of isolates of all fungi}} \times 100$$

4. Test fungi

Aspergillus flavus was selected as main test fungus due to its higher relative density and well known aflatoxigenic potency. Other eight (08) fungal taxa *viz*. *Alternaria* sp., *Aspergillus fumigatus*, *Aspergillus Niger*, *Aspergillus terreus*, *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp. and *Penicillium* sp. isolated during mycological analysis were selected to determine fungitoxic spectrum of essential oil.

5. Extraction of *Ocimum basilicum* essential oil

The leaves of *Ocimum basilicum* were collected from the Botanical Garden, Deen Dayal Upadhyaya Gorakhpur University, and Gorakhpur for the extraction of *O. basilicum* essential oil (OBEO). Leaves (500g) were thoroughly washed and subjected to Clevenger's hydrodistillation apparatus for three hours. The hydrophobic portion (CCEO) was collected, its water traces removed using sodium sulphate and then stored in dark clean glass vial at 4±2°C [15].

6. Antifungal activity of OBEO

The fungitoxic efficacy of OBEO against isolated *A. flavus* in terms of minimum inhibitory concentration (MIC) was determined using poisoned food technique [9]. Different concentrations *viz*. 0.5, 1.0, 1.5, 2.0, and 2.5 µl/ml of OBEO were prepared using 0.5 ml 5% tween-20 and then mixing it with 9.5 ml of PDA medium in presterilized Petri dishes (90mm). The control sets without OBEO were also kept parallel as reference. All the poured Petri dishes were aseptically inoculated with 5 mm diameter disc of *A. flavus* followed by incubated at 27±2°C for 6-7 days. After incubation, the radial growth of fungal colony of treatment and control sets was measured and percent mycelia inhibition was calculated [14].

$$\text{Mycelia inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where,

C = radial growth of fungus in control (mm); T = radial growth of fungus in treatment (mm)

7. Fungitoxic spectrum of OBEO

The spectrum of fungitoxicity of the OBEO was determined against 08 isolated storage fungi during mycological analysis of groundnut sample *viz*. *Alternaria* sp., *Aspergillus fumigatus*, *Aspergillus Niger*, *Aspergillus terreus*, *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp. and *Penicillium* sp. at MIC (2.0 µl/ml) and 2MIC (4.0 µl/ml) against *Aspergillus flavus* by usual poisoned food technique using PDA [9].

8. *In situ* fumigant activity of OBEO against *Aspergillus flavus*

To determine the fumigant antifungal efficacy of OBEO during storage, 100 g of groundnut seeds were kept separately (three sets) in different plastic containers having aerial volume 250 ml. Each container was inoculated with 1 ml spore suspension (≈10^6 spores/ml) of *A. flavus*. Two sets of containers were fumigated separately with OBEO having concentrations 2.0 µl/ml and 4.0 µl/ml (v/v) i.e. MIC and 2MIC against *A. flavus*, while one set of container run parallel as control without OBEO treatment. All the containers were sealed and stored for six months at room temperature i.e. 27±2ºC [14, 16]. After storage, mycological analysis of fumigated groundnut seeds for only *A. flavus* extent was performed [11].

Result and Discussion

The procured groundnut seeds studied in the present study had a pH of 6.71 and relatively high moisture content (23.82%) suggesting favourable conditions for the development of storage fungi and rapid biodeterioration during storage. Moisture content is one of the most important elements affecting the growth of storage fungi in oilseeds. High moisture content promotes seed respiration, enzymatic activity, lipid degradation and microbial colonization [17, 18]. High moisture contents in groundnut seeds make them more susceptible to fungal invasion and aflatoxin contamination during storage, as described by Mutegi *et al*. [19], Yeboah *et al*. [20], Aminou *et al*. [21] etc. The slightly acidic pH of the seeds may also have favoured the growth of fungi, as most storage fungi, especially the *Aspergillus* and *Penicillium* species, do well in mildly acidic environments [22].

Table 1: pH and moisture content (%) of collected groundnut seeds

Parameter	Groundnut seed sample
pH	6.71±0.03
Moisture content (%)	23.82%

Mycological study of stored groundnut seeds indicated the presence of 1194 fungal isolates belonging to six different genera with some unexplained forms indicating substantial fungal contamination of the stored seeds. The dominant fungal flora consisted of *Aspergillus* species like *A. flavus*, *A. fumigatus*, *A. Niger* and *A. terreus*. Similar predominance of *Aspergillus* spp. in stored oilseeds was found by Kakde and Chavan [23], Aiswarya *et al*. [24] and Guo *et al*. [25]. Aspergilli are regarded typical storage fungi due to their capability to develop at low water activity and high temperature conditions that are usually seen during storage. The highest relative density among the isolated fungus was recorded for *A. flavus* (25.29%) followed by *Penicillium* spp. (18.25%) and *A. Niger* (13.14%) (Table 2; Figure 1). The dominance of *A. flavus* is very important since this fungus is known to produce aflatoxins, especially aflatoxin B1, one of the most hazardous natural carcinogens [26, 27]. The frequent prevalence of *A. flavus* in stored groundnut seed and its relationship with aflatoxin contamination have also been reported in previous research [1, 28].

Table 2: Mycoflora analysis of collected stored groundnut seed samples

Isolated fungi	No. of fungal isolates	Relative density %
Alternaria sp.	36	3.02
Aspergillus flavus	302	25.29
Aspergillus fumigatus	58	4.85
Aspergillus niger	157	13.14
Aspergillus terreus	141	11.81
Curvularia sp.	85	7.12
Fusarium sp.	55	4.61
Mucor sp.*	-	-
Penicillium sp.	218	18.26
Rhizopus sp.*	-	-
Unidentified	42	3.52
Total isolates	1194	

*Members of order Mucorales are not included

The second most frequent group of fungi isolated from the seeds was *Penicillium* spp. *Penicillium* spp. are essential storage fungi that can cause seed discolouration, loss in germination potential and generation of mycotoxins such as citrinin [29]. The high incidence of *Penicillium* spp. in the present investigation signifies inadequate storage hygiene and excess moisture situations. Another prominent spoilage fungus related with deterioration of stored food commodities was *Aspergillus Niger*, recorded with 13.14% relative density. *Alternaria* spp. recorded the lowest relative density (3.01%) followed by unidentified fungi (3.51%) (Table 2). The low incidence of *Alternaria* spp. could be related to poor adaption to xerophilic storage conditions than *Aspergillus* and *Penicillium* species. Similar tendencies were seen by Kumar *et al*. [14], Aiswarya *et al*. [24], Guo *et al*. [25] and Prasad *et al*. [30].

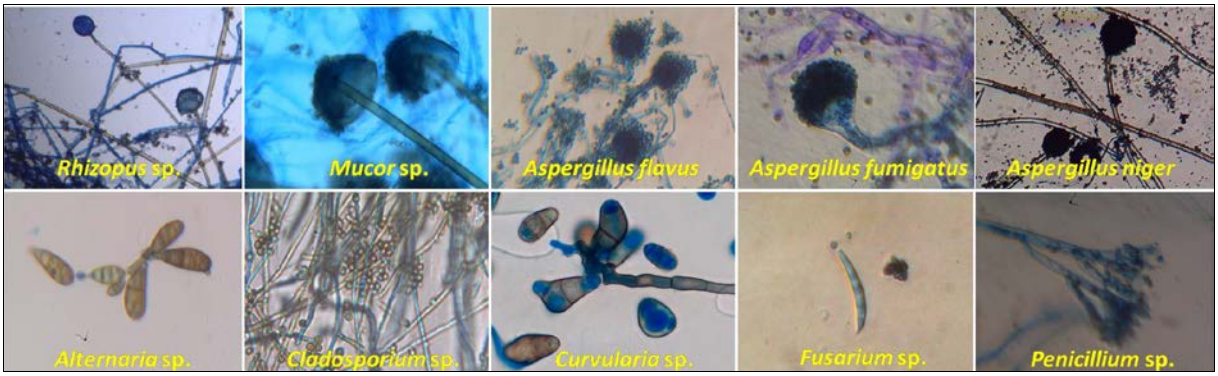

Fig 1: Some storage fungi (microscopic) isolated from groundnut seeds

The present study revealed that the OBEO exhibited significant antifungal efficacy against *A. flavus* and other storage fungi associated with groundnut seeds. The MIC of OBEO against *A. flavus* was found at 2.0 µl/ml, demonstrating significant fungitoxic efficacy even at lower concentration (Table 3; Figure 2). Similar antifungal activity of basil essential oil against *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus* species have been reported by Kumar *et al*. [31], Saggiorato *et al*. [32], Nganou *et al*. [33] and Kaur *et al*. [34]. The broad fungitoxic spectrum of OBEO obtained against eight storage fungi isolated from groundnut seeds (Table 4) and its fumigant fungitoxic efficacy (Table 5) indicates its potentiality as a natural antifungal agent for seed preservation.

Table 3: Antifungal activity of *Ocimum basilicum* essential oil against *A. flavus* using poisoned food technique

Concentration (µl/ml)	Colony diameter (cm)	Percent inhibition
Control	7.40±0.46^{c}	0.00 ±0.00^{a}
0.50	2.63± 1.01^{b}	64.76±11.69^{b}
1.00	0.67± 0.15^{a}	91.05±1.54^{c}
1.50	0.17± 0.06^{a}	97.77±0.67^{c}
2.00	0.00± 0.00^{a}	100.00±0.00^{c}
2.50	0.00± 0.00^{a}	100.00±0.00^{c}

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

The antifungal activity of OBEO may be due to the presence of physiologically active volatile chemicals such linalool, eugenol, methyl eugenol, cineole, camphor and terpinenes [31, 35]. These chemicals are known to destabilize the integrity of the fungal cell wall and membrane, interfere with mitochondrial respiration, hinder spore germination and modulate enzyme activity leading to the fungal cell death [5, 36, 37]. The efficacy of essential oils as eco-friendly alternatives to synthetic fungicides in management of storage fungi and avoidance of mycotoxin contamination was also highlighted by previous researchers including Tripathi and Dubey [38], Kumar *et al*. [31] and Mishra *et al*. [11].

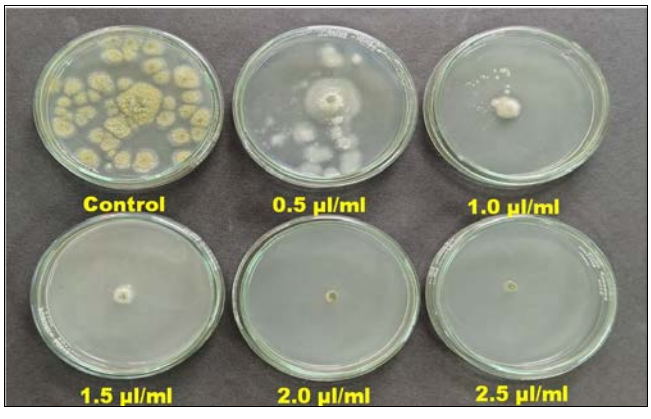

Fig 2: Antifungal efficacy of OBEO against *Aspergillus flavus*

Table 4: Fungitoxic spectrum of OBEO against some isolated storage fungi

Fungi	Percent inhibition (%)	
	MIC (2.0 µl/ml)	2×MIC (4.0 µl/ml)
Alternaria sp.	88.36	100
Aspergillus fumigatus	100	100
Aspergillus niger	100	100
Aspergillus terreus	100	100
Cladosporium sp.	76.91	100
Curvularia sp.	92.18	100
Fusarium sp.	100	100
Penicillium sp.	100	100

Table 5: Per cent inhibition in *A. flavus* isolates after six months fumigation of OBEO

Samples	No. of *A. flavus* isolates	Per cent inhibition
Control	674	-
Fumigation at MIC (2.0 µl/ml)	334	50.45
Fumigation at 2×MIC (4.0 µl/ml)	283	58.01

Physicochemical evaluation of OBEO indicated the colour of the oil to be pale yellow to yellowish green and aroma to be slightly peppery, sweet and clove-like which is in close agreement with the earlier findings [39, 40]. These physicochemical characteristics are indicative of the quality and purity of the essential oil. Essential oils are biodegradable, non-persistent, environmentally friendlier and less harmful to human health compared to synthetic fungicides [5, 31, 41, 42]. Therefore, the present study gives solid evidence for the use of OBEO as a natural phytopreservative for protecting stored groundnut seeds from fungal infestation and improving the storage quality and safety.

Conclusion

The present study revealed that the stored groundnut seeds are highly susceptible to fungal infestation mainly by *Aspergillus flavus* along with several other species storage fungi under high moisture conditions. OBEO showed high potency along with broad range antifungal activity with good suppression of storage fungi. Therefore, OBEO can be an effective eco-friendly and natural phytopreservative for improving storage quality and safety of groundnut seeds.

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