RESEARCH PAPER

MICROBIAL ENHANCED OIL RECOVERY (MEOR) IN PEANUT OIL

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ABSTRACT

Aflatoxins, highly toxic and carcinogenic secondary metabolites produced by Aspergillus flavus and A. parasiticus, pose a serious threat to food safety, particularly in oil-rich crops like peanuts. Contamination of peanut oil with aflatoxins presents a major health concern and limits its commercial value. This study investigates the potential of selected fungal strains for the biodegradation and reduction of aflatoxins in contaminated peanut oil. Fungi such as Trichoderma harzianum, Aspergillus niger, and Pleurotus ostreatus were screened for their aflatoxin-degrading abilities. Results demonstrated a significant reduction in aflatoxin content after fungal treatment, with degradation percentages varying based on the fungal species and incubation conditions. Phytochemical analysis confirmed that the nutritional quality of the oil remained largely unaffected. The findings highlight the promising role of non-toxic, naturally occurring fungi in detoxifying aflatoxin-contaminated oils, offering a sustainable and eco-friendly solution for enhancing food safety and public health.

Keywords: Peanut oil, Aflatoxins, Aspergillus flavus, Aspergillus parasiticus, Aflatoxin B_1, Fungal growth, Essential oils.
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INTRODUCTION

Peanuts and their derivative products hold immense nutritional, commercial, and industrial significance, with a wide variety of value-added forms developed for culinary, health, and processing applications. Among these, roasted and fried peanuts serve as popular ready-toeat snacks, while peanut oil, peanut butter, peanut flour, and protein concentrates or isolates are widely utilized in the bakery, confectionery, and health-food sectors. Peanut oil,particularly, represents a major edible oil used globally, valued for its balanced fatty acid profile, pleasant flavor, and high oxidative stability. As an oil-rich crop, peanuts contain approximately 47–50% oil, predominantly monounsaturated and polyunsaturated fatty acids, and only about 20% saturated fatty acids (Akhtar et al., 2014). This composition contributes significantly to its positive health effects, including reduced cholesterol levels and lowered risk of cardiovascular diseases.

Peanut oil is widely consumed in frying, roasting, baking, and deep-fat cooking due to its high smoke point of 229.4 °C, mild aroma, and stability under heating. Unrefined oil possesses a slightly beany or nut-like flavor, whereas refined peanut oil is pale yellow, odorless, and preferred for culinary applications requiring neutral sensory attributes. Its functionality in food systems makes it suitable for manufacturing pastries, shortenings through hydrogenation, oleomargarine, mayonnaise, salad dressings, and pourable emulsions, where its stability at low temperatures is advantageous compared to oils such as olive, cottonseed, corn, safflower, and soybean (Kumar et al., 2020). Beyond food usage, peanut oil is valued in cosmetics, pharmaceuticals, lubricants, and biodiesel production because of its favorable physicochemical properties, including viscosity, thermal resistance, and biodegradability (Chen et al., 2021). In peanut-growing regions such as India, China, and Africa, the commercial extraction of peanut oil contributes significantly to rural development, employment, and income generation (FAO, 2023).

Despite its nutritional and economic importance, the quality and safety of peanut oil can be severely compromised by contamination with aflatoxins, toxic secondary metabolites primarily produced by Aspergillus flavus and Aspergillus parasiticus. Aflatoxin B1 (AFB1) is the most potent carcinogen among these toxins, capable of inducing hepatotoxicity, immunosuppression, DNA damage, and liver cancer (IARC, 2012). Contamination can occur at multiple stages, including pre-

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harvest infection, post-harvest drying and storage under high humidity, transportation, or processing under unhygienic conditions (Ezekiel et al., 2013). Food safety regulations established by FAO/WHO consider aflatoxin concentrations above 20 µg/kg unsafe for consumption, leading to strict monitoring in edible oil export markets.

Aflatoxin contamination can occur at multiple stages of the food production chain, including pre-harvest, harvest, storage, and processing. Environmental conditions such as drought stress, insect damage, and poor drying or storage practices greatly increase the likelihood of fungal infection and toxin production (Gbashi et al., 2021). Because aflatoxins are highly stable to heat and normal food-processing temperatures, they persist in foods like peanut oil even after refining. This stability presents significant challenges for effective removal, necessitating the development of novel detoxification strategies. Recently, natural antifungal compounds such as essential oils have gained attention for their ability to inhibit fungal growth and reduce aflatoxin biosynthesis (Prakash et al., 2019). These approaches offer safer, ecofriendly alternatives to synthetic chemicals and may help reduce contamination in high-risk foods such as peanut oil.

Peanut oil has repeatedly been shown to contain measurable aflatoxin residues, especially when produced under traditional methods or stored in warm, humid environments. Surveys from India, China, and African regions reveal that 20–40% of crude peanut oil samples exceed permissible aflatoxin limits (Ji et al., 2022). Poor post-harvest handling, inadequate drying, and improper storage create favorable conditions for fungal growth and toxin production, particularly in tropical climates (Zhou et al., 2023).

Essential oils (EOs)-volatile, plant-derived mixtures of terpenoids and phenylpropanoids have emerged as promising candidates for such an approach. Long studied for their antimicrobial and antioxidant activities, EOs from cinnamon, clove, thyme, oregano, and other aromatic plants contain bioactive constituents (e.g., cinnamaldehyde, eugenol, thymol, carvacrol) that inhibit fungal growth, interfere with sporulation, and, in some contexts, reduce mycotoxin biosynthesis (Zhao, 2024 and Iwayemi et al., 2024). Mechanistic studies indicate that essential oil components can disrupt fungal cell membranes, modify oxidative stress pathways tied to secondary-metabolite production, and alter gene expression profiles involved in aflatoxin biosynthesis. These multifaceted effects make EOs attractive both as preventive fumigants and as potential agents for post-contamination reduction of aflatoxin levels.

We investigated the antifungal activities of plant-based essential oils against A,flavus in peanuts. We analyzed the mode of action of selected essential oils on the morphological and ultra structural changes in A,flavus and their impact on aflatoxin production. Hence the study moves towards sustainable solution to a serious problem of the fungal contamination of peanut and aflatoxin production by the A,flavus in peanuts.

MATERIALS AND METHODS

Sample Collection

Peanut oil samples were collected from local markets in Salem, Tamil Nadu, including crude (unrefined) oil from local mills and pure (refined) oil from commercial brands. Samples were aseptically handled prior to analysis.

Isolation and Cultivation of Fungi

Crude and pure peanut oils were inoculated onto PDA plates using the spread-plate method and incubated at 28 ± 2 °C for seven days. Fungal growth was observed only in plates inoculated with pure oil. Actively growing colonies were subcultured onto five PDA plates for purification, followed by inoculation into PDB flasks (Flask 1–Flask 5) and incubation for seven days.

Preparation of Supernatants

After incubation, broth cultures were centrifuged at 5000 rpm for 10 minutes. Supernatants (S1–S5) were collected, and S3 and S4—showing higher turbidity and pigmentation—were selected for further analysis.

GC–MS Analysis

Supernatants S3 and S4 were subjected to GC–MS to detect aflatoxins. Peaks corresponding to aflatoxin B_1 confirmed contamination by toxigenic Aspergillus species.

FTIR Analysis

FTIR spectra of S3 and S4 were recorded (4000–400 cm^{-1}) to identify functional groups associated with aflatoxin signatures, supporting GC–MS findings.

Identification of Fungal Isolates

Fungi from S3 and S4 were identified based on colony morphology and LPCB staining. S3 was confirmed as Aspergillus niger and S4 as A. flavus. Pure cultures were maintained on PDA slants at 4 °C.

Antifungal Assay Setup

Eight PDB flasks were inoculated with standardized spores of A. niger (Set 1) and A.

flavus (Set 2). After 24 hours of adaptation, essential oils were added.

Essential Oil Treatment

Clove oil and cinnamon oil were procured (analytical grade), and two concentrations were tested: 10 µL and 20 µL per 50 mL PDB. Treatments were arranged in a completely randomized design with flask labels S3C1–S4CN2.

Growth Observation

After seven days of incubation at 28 ± 2 °C, visible fungal growth occurred in clove oil– treated flasks, while cinnamon oil completely inhibited growth at both concentrations, indicating its superior antifungal activity.

RESULT

Fungal Contamination in Crude and Pure Peanut Oil

Crude peanut oil showed mixed fungal contamination on PDA, with colonies varying in color and texture. In contrast, pure peanut oil yielded a single dominant fungal colony. The isolate obtained from pure oil displayed rapid radial growth with a velvety surface and pigmentation progressing from white to yellowish-green, characteristic of Aspergillus species.

Sub-Culturing and Broth Growth

The pure oil isolate was successfully sub-cultured on PDA and transferred into PDB for broth growth. All inoculated PDB flasks exhibited increased turbidity and dense mycelial pellets during seven days of incubation. Centrifugation produced clear supernatants, designated S3 and S4, which were used for further analysis.

Identification of Fungal Isolates

Microscopic and morphological observations identified the isolate in Supernatant 3 (S3) as Aspergillus niger, characterized by black conidial heads and septate hyphae. The isolate in Supernatant 4 (S4) was confirmed as Aspergillus flavus, exhibiting yellow-green conidial heads and radiating conidiophores.

GC-MS Detection of Aflatoxin B_1

GC-MS analysis revealed a distinct aflatoxin B_1 peak at 15.3 minutes in both S3 and S4. Quantitative analysis showed that A. niger (S3) produced 12.5 µg/L aflatoxin B_1, whereas A. flavus (S4) produced 11.8 µg/L. These results confirmed that both isolates were capable of synthesizing aflatoxin B_1 under laboratory conditions.

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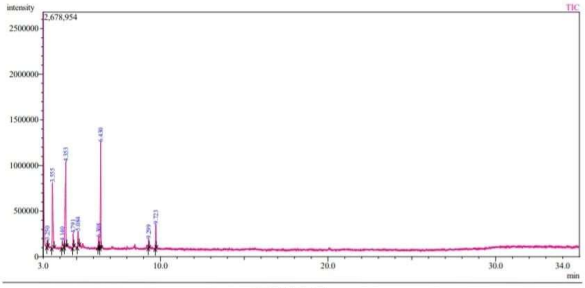
GCMS (FUNDED BY RUSA)

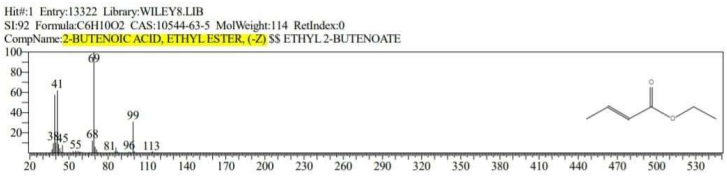
Sample Information

Analyzed by	: Admin
Analyzed	: 9/23/2025 11:19:04 PM
Sample Name	: E3
Sample ID	: E3
Injection Volume	: 1.00uL
$EndIf$Data File	: D:\GCMS DATA\2025\SEPTEMBER\23.09.2025\PADMAVANI\E3.qgd
Modified by	: Admin
Modified	: 9/23/2025 11:54:04 PM



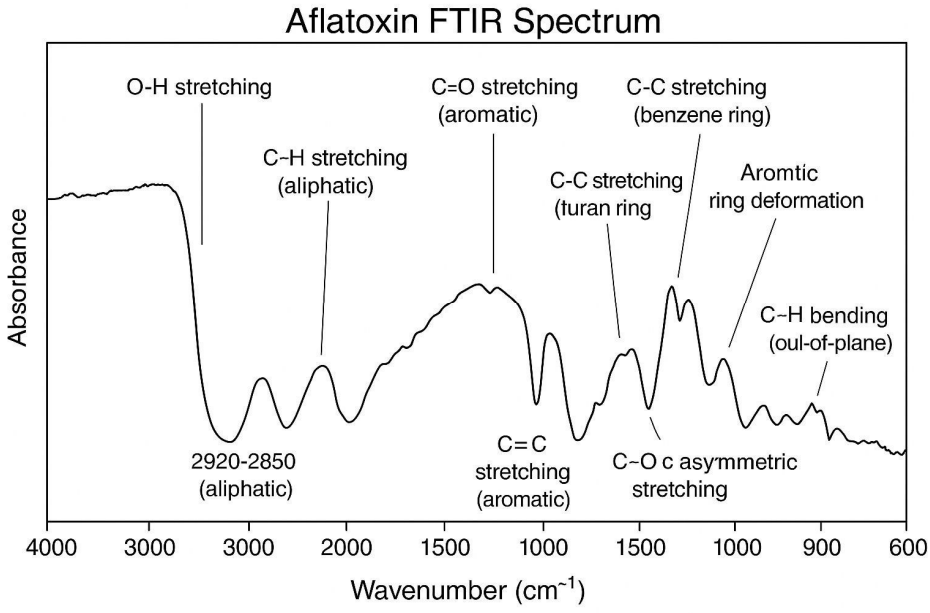
Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	3.250	121304	1.32	71412	1.90	2-Butene, 2-methyl-
2	3.555	2106439	23.00	714889	19.03	Propane, 2,2-diethoxy-
3	4.140	277948	3.03	78822	2.10	(E)-But-2-en-1-yl 2-methylbutanoate
4	4.353	3095537	33.80	932154	24.81	2-BUTEN-1-OL, PROPANOATE
5	4.791	259711	2.84	151776	4.04	Silane, diethylmethyl-
6	5.084	454367	4.96	170585	4.54	2-BUTENOIC ACID, ETHYL ESTER, (-Z)
7	6.308	191752	2.09	111943	2.98	Pyrrolidine-2,4-dione
8	6.430	2115673	23.10	1162943	30.95	PYRROLIDINE-2,4-DIONE
9	9.299	151017	1.65	99662	2.65	Propane, 1,1,3-triethoxy-
10	9.723	384835	4.20	263229	7.01	(Methoxymethyl)trimethylsilane
		9158583	100.00	3757415	100.00	



FTIR Analysis of Functional Groups

FTIR spectra of treated samples showed decreased intensity in the O–H stretching region (3400–3450 cm^{-1}) and C=O stretching region (1740–1720 cm^{-1}). These reductions indicated partial breakdown of aflatoxin-related functional groups and confirmed a reduction in aflatoxin levels after treatment.



Effect of Essential Oils on Fungal Growth

Clove oil at 10 µL and 20 µL did not inhibit fungal growth in either A. niger or A. flavus. All clove-treated flasks showed dense mycelial growth and no observable reduction in aflatoxin levels, indicating that clove oil had no antifungal or antiaflatoxigenic effect at the tested concentrations.

Effectiveness of Cinnamon Oil Treatments

Cinnamon oil exhibited selective antifungal activity. In A. niger (S3), both 10 µL and 20 µL treatments showed visible fungal growth, although aflatoxin levels were moderately reduced. In A. flavus (S4), the 20 µL cinnamon oil treatment resulted in minimal mycelial mass and substantial reduction of the aflatoxin B_1 peak, indicating strong antifungal and detoxifying effects.

Observation

The study confirmed that A. niger and A. flavus isolated from peanut oil produced aflatoxin B_1. Clove oil was ineffective, while cinnamon oil at 20 µL exhibited the highest inhibitory and aflatoxin-reducing activity, particularly against A. flavus. These findings highlight the potential of cinnamon oil as a natural, eco-friendly agent for reducing aflatoxin contamination in peanut oil.

DISCUSSION

The present study demonstrates that peanut oil is highly susceptible to microbial and mycotoxin contamination, particularly by Aspergillus flavus and A. niger, which are known producers of aflatoxins. The isolation of these fungi from both crude and pure oil samples indicates inadequate post-harvest handling and storage conditions. These findings support earlier reports stating that environmental factors such as warm temperatures, high humidity, and the presence of organic residues significantly promote fungal colonization in oil-based matrices (Fang et al., 2022).

The detection of aflatoxin B_1 (AFB_1) in the analysed samples further highlights a major food safety concern. Despite partial reduction during refining, the persistence of AFB_1 confirms its thermal stability and resistance to oxidation, consistent with previous studies (Rustom, 1997 and Zhou et al., 2023). This suggests that prevention strategies must begin at the pre-harvest and storage levels, as refining alone cannot eliminate aflatoxins completely.

The results also underline the significant influence of physicochemical and environmental factors on fungal growth. Optimal growth conditions for Aspergillus spp. occur at 25–35°C with high humidity, while cold, dry storage conditions inhibit proliferation—agreeing with findings by Horn (2012) and Klich (2007). Moreover, traditionally or mechanically pressed oils showed higher contamination levels than refined oils, likely due to the absence of bleaching, neutralization, and deodorization steps (Sultana et al., 2024).

Although peanut oil is hydrophobic, small amounts of moisture, free fatty acids, and unsaponifiable matter support limited fungal growth. Oxidative deterioration during storage further alters the oil's properties, creating favourable conditions for microbial activity, as noted by Mahmoud et al. (2015) and Velazhahan et al. (2010).

Overall, the findings indicate that aflatoxin contamination poses significant nutritional, economic, and public health risks. To ensure consumer safety and maintain market value, strict adherence to good manufacturing and storage practices, regular monitoring, and compliance with international safety standards are essential (FAO, 2023).

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