

Original Research Article

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Screening of Aflatoxin and Aspergillus Contamination in Selected Nuts and Dry Fruits

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ABSTRACT

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The present study investigated the possible presence of aflatoxins in six raw-consumed nuts and dry fruits—almond, broken cashew, walnut, groundnut, pistachio, and raisin—collected from local markets in Chennai. Samples were analysed for fungal contamination and aflatoxins using fungal culture, TLC, and UV-spectrophotometric methods. No fungal growth was initially observed on the serially diluted SDA plates; however, fungal growth was detected in the 10⁻¹ dilution tubes of four samples. Based on macroscopic and microscopic characteristics, *Aspergillus niger* and *Aspergillus flavus* were identified. TLC analysis showed fluorescence only in the broken cashew and pistachio extracts, while no characteristic aflatoxin-associated spots were observed in the fungal extracts. Spectrophotometric analysis at 360 nm and 280 nm showed no significant absorption peaks. Aflatoxin levels were not detectable under the analytical conditions used and were therefore considered to be below the detection limit of the method. The fungal contamination observed may be associated with handling or storage conditions; however, these factors were not directly investigated. Further confirmation using sensitive methods such as HPLC or LC-MS/MS is recommended.

Introduction

Mycotoxins are secondary metabolites produced by fungi and represent important contaminants of food and feed, causing mycotoxicosis following ingestion of preformed fungal toxins, while poisoning caused by consumption of toxic mushrooms is termed mycetismus. Their production is influenced by factors such as substrate, pH, moisture and temperature, and commonly affects cereals, nuts, dried fruits and spices (Hope *et al.*, 2005; Heydt *et*

al., 2010; Gallo *et al.*, 2016). Major food-associated mycotoxins include aflatoxins, ochratoxin A, trichothecenes, fumonisins and patulin, with aflatoxins produced mainly by *Aspergillus flavus* and related species and occurring in commodities such as groundnuts, cereals, oilseeds, spices and other agricultural products; aflatoxin M₁ may also occur in milk following exposure to contaminated feed. Among these, aflatoxins—particularly B₁—are of major toxicological concern because of their hepatotoxic,

carcinogenic and mutagenic effects, with exposure associated with impaired growth, liver injury and other adverse health effects. The clinical condition caused by aflatoxins is classified under Mycotoxicoses category. They are found to cause infections mostly to the human beings. The effects of this clinical condition would range from a delayed or stunted growth to acute hepatic necrosis or liver cirrhosis. While, other conditions like alteration in digestion, oedema, coma, and nutrient absorption variance etc., are also reported. Aflatoxin production is favoured under suitable temperature and moisture conditions, and several analytical methods, including TLC, HPLC, LC-MS, ELISA, immunoassays, biosensors, near-infrared spectroscopy and hyperspectral imaging, have been developed for their detection (Yao *et al.*, 2015). Thin-layer chromatography (TLC) has been widely used for screening and detection because of its simplicity and applicability to food samples. This method has been regarded as the technique of choice since 1960 by the Association of Official Analytical Chemist, Considering the potential health risks associated with aflatoxin contamination and the limited information available for certain food commodities in South India, this study was focused on the detection of aflatoxins in raw food samples collected from different markets in Chennai, India, thereby contributing to the assessment of food safety and potential consumer exposure.

Materials and Methods

Materials

Materials included standard microbiological glassware, Sabouraud Dextrose Agar (SDA), Lactophenol Cotton Blue, 0.85% sterile saline, chloroform, methanol and silica gel TLC plates, along with a microscope, UV transilluminator, spectrophotometer, mechanical shaker, micropipettes, blender, inoculation loop and Whatman No. 1 filter paper. Six raw food samples—almond, broken cashew, walnut, groundnut, pistachio and raisin—were collected from different market/sales points and processed aseptically.

Methodology

Fifty grams of each sample as listed in Table -1, was ground to a coarse powder, while raisins were ground to a paste, and serial dilution was performed using 0.85% sterile saline. The prepared saline was autoclaved at 121°C for 15 min, and samples were serially diluted up

to 10⁻⁷. For fungal isolation, 0.1 mL of appropriate dilutions was spread on SDA supplemented with chloramphenicol, followed by incubation in a moist chamber for 48 hours per the procedure adopted by Kutama *et al.*, 2022. Samples showing fungal growth as in the fig: 1 & 2, were re-inoculated, and distinct colonies were subcultured on SDA to obtain pure cultures shown in the fig: 3& 4. Macroscopic and microscopic characteristics were examined, and Lactophenol Cotton Blue staining was used for fungal identification and were listed in Table - 2. For aflatoxin detection, 10 g of each ground sample was extracted with 100 mL chloroform, mixed thoroughly and left covered and undisturbed for 48 hours. The extract was filtered through Whatman No. 1 filter paper and stored under refrigerated conditions.

Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was performed on silica gel slides using a chloroform: phenol (97: 3) solvent system. Ten microlitres (10 µL) of extract was spotted, and the plates were developed in a saturated TLC chamber, dried and examined under a UV transilluminator at 365 nm for characteristic fluorescence as depicted in fig: 9, 10, 11 and 12 indicative of aflatoxins. UV-visible spectrophotometric analysis was additionally performed on the sample extracts to assess absorbance characteristics associated with aflatoxins. The overall methodology followed previously reported procedures, particularly Kutama *et al.*, (2022) and other cited methods.

UV Absorption Spectrophotometry

The method was adopted with reference to Espinosa *et al.*, 2011; Qureshi and Javed 2021. Sample extracts for spectrophotometric analysis was obtained for all the 6 samples and from that of the colonies grown on the SDA plates by mixing them with chloroform and leaving the setup undisturbed for 48 hours. The extract is then filtered using filter paper and subjected to spectroscopic analysis at the absorption range of 360nm as aflatoxins are found to exhibit maximum absorption at this wavelength and at the range of 280nm, where AFB1 is found to be hyper chromic.

Results and Discussion

A total of six samples of selected nuts and dry fruits were analysed for fungal contamination. Following serial

dilution, the samples plated on Sabouraud Dextrose Agar (SDA) showed no visible colonial growth. This finding differed from the observations reported by Kutama *et al.*, (2022), who reported the occurrence of fungal contaminants in similar food samples. The absence of growth in the plated serially diluted samples may be associated with differences in the level of fungal contamination, sample characteristics, or experimental conditions.

Despite the absence of colonial growth in the plated serial dilution samples, fungal growth was observed in the 10^{-1} dilution tubes of four samples after four days of incubation. The tubes exhibiting growth were subsequently plated onto SDA for further isolation and identification of the fungal organisms. Based on their macroscopic and microscopic characteristics as described in the Table: 2, two fungal species, *Aspergillus flavus* and *Aspergillus niger*, were identified.

The identified fungal species were distributed equally among the contaminated samples. *Aspergillus niger* was detected in the almond and walnut samples as depicted in figures 3,5&6, whereas *Aspergillus flavus* was detected in the pistachio and groundnut samples as shown in figures 4,7 & 8. The detection of *A. flavus* and *A. niger* in the present study is in agreement with the findings reported by Kutama *et al.*, (2022), who also documented the presence of these *Aspergillus* species in nuts and/or dried food products.

The occurrence of *Aspergillus* species in nuts and dry fruits is of particular importance because these fungi are commonly associated with stored food commodities and may proliferate under favourable environmental conditions. In particular, *A. flavus* is of food-safety significance because certain strains are capable of producing aflatoxins.

Therefore, the detection of *A. flavus* in the pistachio and groundnut samples highlights the need for appropriate storage and handling practices to minimise fungal contamination and potential mycotoxin-related risks.

TLC and Spectrophotometric Results

Thin-layer chromatography (TLC) of extracts obtained from the six samples, using a chloroform: methanol solvent system, showed variable fluorescence under UV illumination, with visible spots observed in selected extracts. However, TLC analysis of the pure fungal

isolates did not reveal a distinct spot corresponding to an aflatoxin standard. Therefore, the TLC findings did not provide definitive evidence for the presence of aflatoxin in the tested fungal isolates.

Similarly, the UV absorption spectra obtained from the six sample extracts and the pure fungal isolates did not exhibit a definitive absorption peak as depicted in Figures 13 & 14, corresponding to the expected aflatoxin signal. Thus, under the experimental conditions employed, neither TLC nor UV spectrophotometric analysis provided conclusive evidence of aflatoxin detection in the analysed samples or isolated fungal cultures.

It should be noted that the absence of a definitive TLC spot or characteristic UV absorption peak does not necessarily establish the complete absence of aflatoxins. Differences in toxin concentration, extraction efficiency, matrix effects, and analytical sensitivity may influence the detection of aflatoxins.

More sensitive and specific analytical techniques, such as high-performance liquid chromatography (HPLC) or validated immunoassay-based methods, may therefore be required for definitive confirmation and quantification.

In conclusion, the recovery of *Aspergillus niger* and *Aspergillus flavus* from the selected nut and dry-fruit samples demonstrates the presence of fungal contamination in the examined materials. Both species are recognized as important food-associated fungi, while *A. flavus* is of particular food-safety significance because certain strains are capable of producing aflatoxins.

In the present study, however, the TLC and UV-spectrophotometric analyses did not provide definitive evidence of aflatoxin production in the examined samples or fungal isolates. The absence of a distinct aflatoxin-associated TLC spot or a characteristic UV absorption peak may be attributable to aflatoxin concentrations below the detection capability of the analytical methods, differences in the toxigenic potential of the fungal isolates, or interference from other compounds present in the sample extracts.

Therefore, the isolation and identification of *A. flavus* should not, by themselves, be interpreted as confirmation of aflatoxin contamination. Detection of the fungus indicates potential risk but does not establish the presence or concentration of aflatoxins.

Table.1 Sample

Sample number	Name of the Sample
1	Almond
2	Broken Cashew
3	Walnut
4	Groundnut
5	Pistachio
6	Raisin

Table.2 Macroscopic and Microscopic morphology of isolated species

Sample No.	Name of the sample	Macroscopic appearance	Microscopic appearance
1	Almond	Immature colonies are covered with white fluffy aerial mycelia and the mature colonies showed salt and pepper effect.	Brownish black globose conidial head , globose vesicle with concave under surface.
3	Walnut	White colonies became black as the culture matured.	Globose vesicle with concave under surface. Sterigmata are produced in two series.
4	Ground nut	Spreading yellow green colonies became green as the culture matured.	Globose vesicle with biserial sterigmata covering the entire vesicle.
5	Pistachio	White colonies velvety in texture became green as the culture matured.	Conidial heads are dark brown and biserial with sub globose vesicle with and conidia covering the entire vesicle.

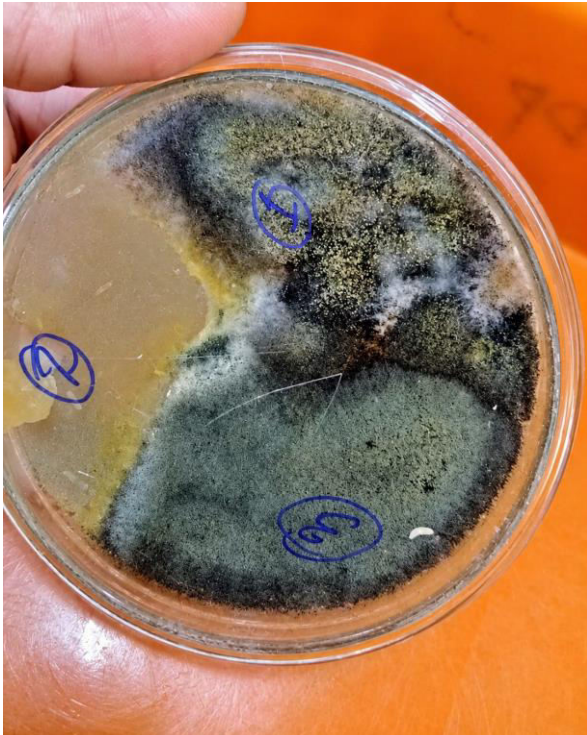


Fig.1 Samples - 1, 2, 3



Fig.2 Samples - 4, 5, 6



Fig.3 Sample 1 & 3 - Pure culture



Fig.4 Sample 4 & 5 - Pure culture



Fig.5 - *Aspergillus niger* - Sample -1



Fig.6 *Aspergillus niger* - Sample -3

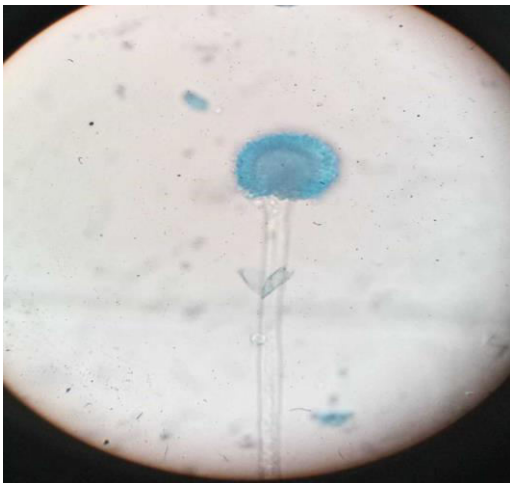


Fig.7 *Aspergillus flavus* - Sample - 4

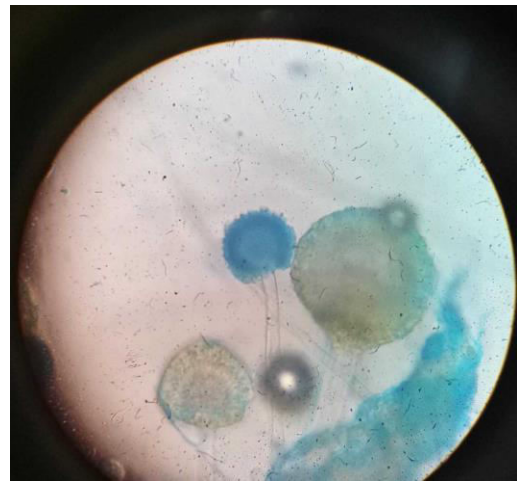


Fig.8 *Aspergillus flavus* - Sample - 5

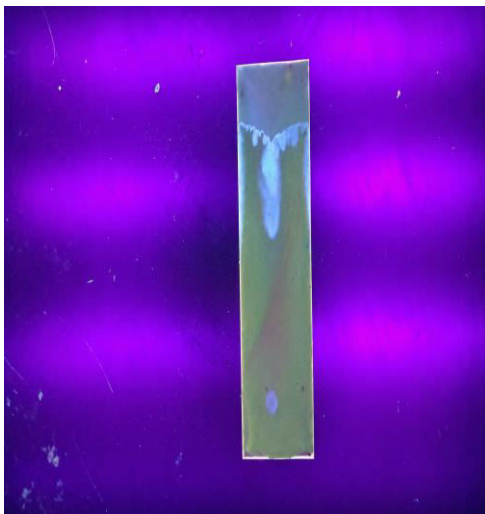


Fig.9 Sample - 1

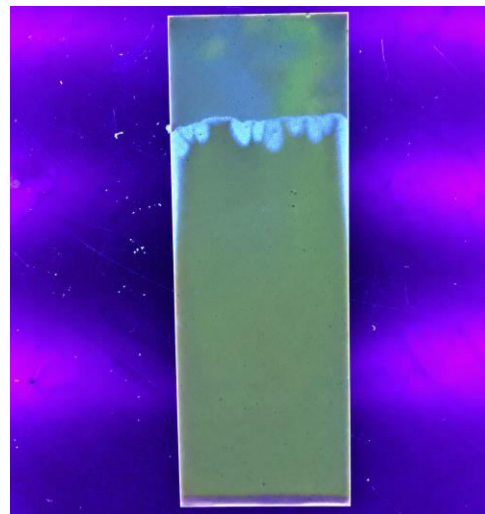


Fig.10 Sample - 3



Fig.11 Sample - 4



Fig.12 Sample -5

Fig.13 UV absorption spectra - 6 samples

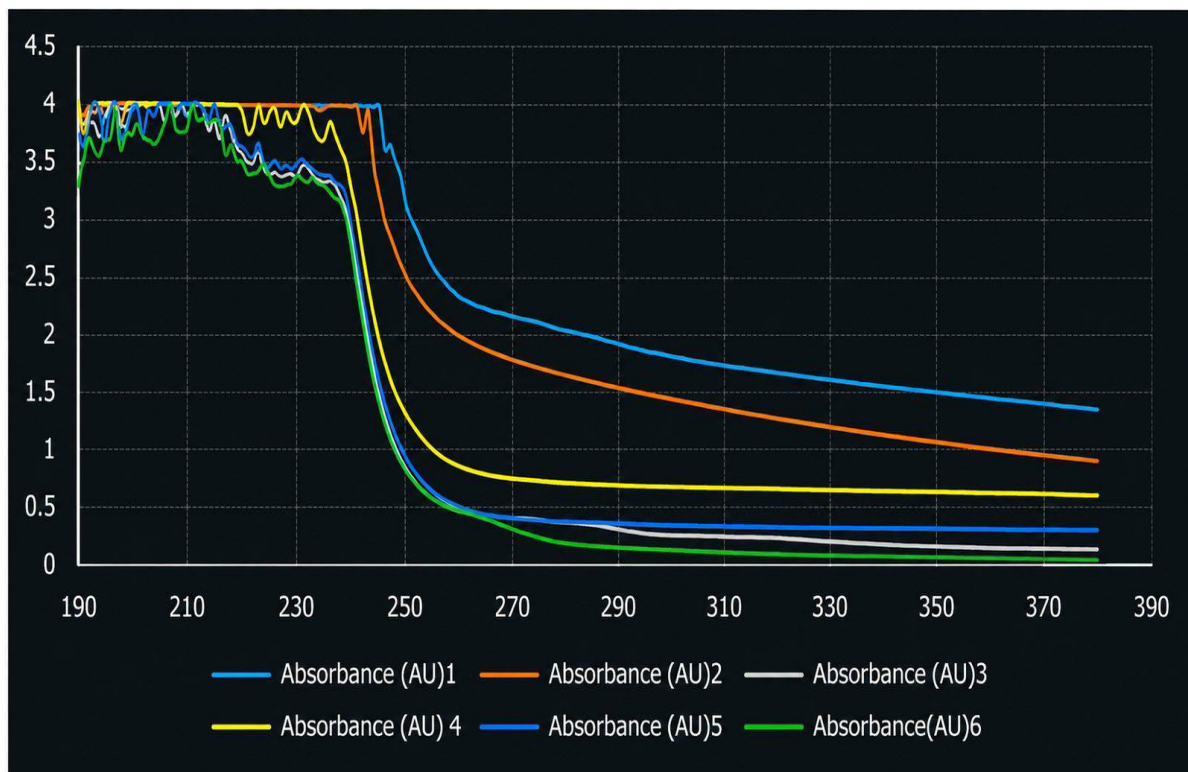
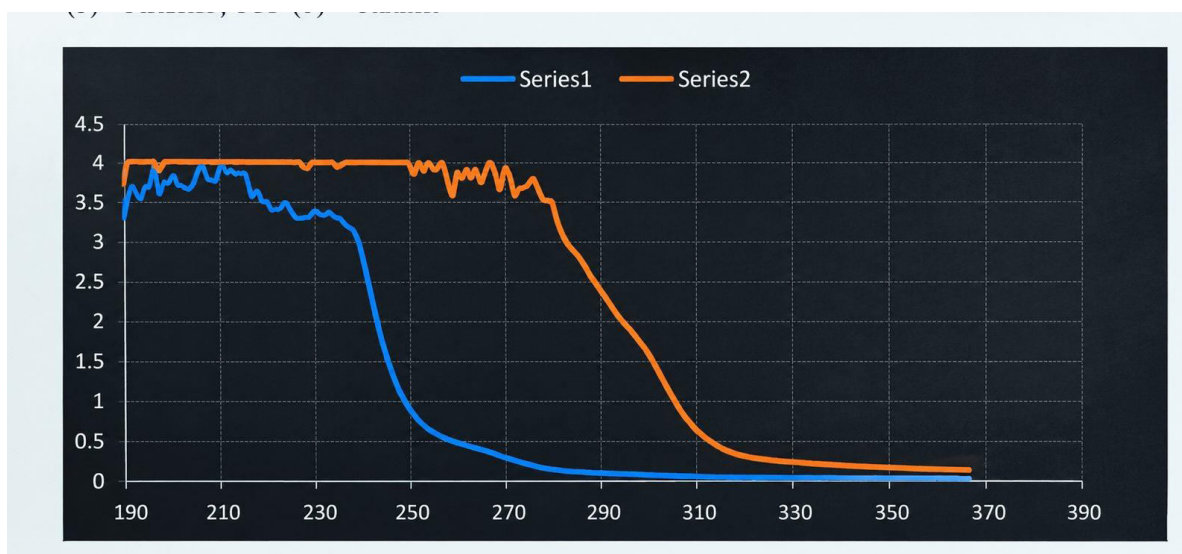


Fig.14 UV absorption spectra of pure culture isolates



More sensitive and specific analytical techniques, such as high-performance liquid chromatography (HPLC) or liquid chromatography–tandem mass spectrometry (LC–MS/MS), would be appropriate for the definitive identification and quantitative determination of individual aflatoxins. These methods would provide more reliable confirmation of aflatoxin contamination and allow assessment of the levels present in the analysed samples.

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Author Contributions

Sowndharya. P: Carried the actual laboratory work, Writing - Original Draft Preparation. C. Soundhari: Developed and refined the experimental protocols, and reviewed the manuscript. D. Prabu: Conceived the idea, Conceptualization, designing the Methodology Research Supervision and analyzed the data.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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