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ORIGINAL RESEARCH ARTICLE

Effect of gamma radiation on fungal load decontamination of marketed spices

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ABSTRACT

In this study, the effects of gamma radiation on the decontamination of fungus, physicochemical properties, and molecular analysis of *Aspergillus* spp. of common spices for storage were evaluated. After being irradiated with gamma doses of 0, 2, 4, and 6 kGy and sealed in glass vials, the spices were stored at room temperature for 180 days. Among the tested spice samples, chili, turmeric, and black pepper powder showed the highest presence of fungal contamination compared to cumin, coriander, garlic, and ginger samples. Microscopy was used to identify a total of 48 isolates, of which 11 were *Mucor*, 25 were *Penicillium*, and 12 were *Aspergillus*. By polymerase chain reaction (PCR) amplification and sequencing of the internal transcribed spacer (ITS) region, a total of 12 Aspergillus genera were identified among them: 5 in black pepper and 7 in red chili. The gamma radiation also reduced the number of microbes compared to the control group. The best gamma radiation doses were found to kill the organisms in the studied spices. These were 6 kGy for red chili, 4 kGy for turmeric and black pepper, and 2 kGy for cumin, coriander, garlic, and ginger. Measurements of the physicochemical parameters were not significantly impacted by the 180-day exposure to gamma radiation; however, the number of fungi drastically decreased. Gamma radiation has been explored as an effective method for decontaminating spices, offering a promising solution for ensuring food safety and quality.

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INTRODUCTION

Since ancient times, spices have been considered aromatic vegetable substances and used as additional components to improve flavor, color, and taste. Spices are grown and harvested in warm and humid areas (Bata-Vidacs *et al*., 2018). The water activity of spices is very high during harvesting and exceeds critical values. An agricultural product contains high moisture in the harvest period, which is a suitable environment for fungal growth (Thanushree *et al*., 2019; Kamal *et al*., 2019). The harvested spices are generally sun-dried in open areas, which may be considered the primary source of fungal contamination, particularly by molds (Molnar *et al*., 2018). Aflatoxigenic fungus contaminates spices as a result of improper harvesting, processing, transportation, and storage (Teng *et al*., 2019). The most common fungi, like *Aspergillus*, *Penicillium*, *Mucor*, and *Rhizopus* were found in spices (Liu *et al*., 2020) and among the fungi, *Aspergillus* produced aflatoxins in the spices (Hammami *et al*., 2014).

Aflatoxin contaminations in spices are a common difficulty worldwide, and many spices waste these contaminations (Yakubu and Vyas, 2020; El Darra *et al*., 2019). Aflatoxins are considered secondary toxic metabolites during regular consumption. People may experience nutritional deficiencies, mutagenic effects, and carcinogenic effects from consuming contaminated spices (Ayelign and De Saeger, 2020). The rate of aflatoxin production in spices depends on fungal strains, substrate, geographic area, climate, and plant culture systems. *A. flavus* strains are found in temperate and tropical regions in soil and cultivated zones (Anelli *et al*., 2019; Dadzie *et al*., 2019). Polymerase chain reaction (PCR) amplification is a good way to find strains of *aflP*, *aflR*, and *aflM* genes from the aflatoxin biosynthesis pathway that produce and do not produce aflatoxin (Gnonlonfin *et al*., 2013; Rao *et al*., 2020). A recent study by Thanushree *et al*., 2019 reported that fungal contamination of spices leads to aflatoxins production at 25-30 °C, moisture contents above 16%, and a corresponding water activity of 0.70. Fungal growth and aflatoxin contamination reduction are necessary for spices. In this case, gamma irradiation is a good way to keep spices from getting contaminated without changing their physical, chemical, or nutritional properties (Molnar *et al*., 2018; Umesha and Manukumar, 2018). Food is exposed to ionizing radiation through gamma radiation, which includes gamma rays produced by artificial sources of the radioisotope Co-60. Microorganisms are unable to develop on food since it is an efficient way to harm organisms' DNA (Schultzhaus *et al*., 2020). Gamma-ray therapy is currently acknowledged on a global scale, and consumer-taken food samples are unaffected (Hassan *et al*., 2019; Esmaeili *et al*., 2018). The research builds on a solid background of food safety concerns related to spices, highlighting the urgent need for innovative decontamination techniques. The novelty of this study lies in its exploration of gamma radiation as a decontamination method for marketed spices, a domain that remains relatively underexplored. By evaluating the efficacy of gamma radiation, this research aims to bridge a critical gap in knowledge and contribute to the development of safer spice products.

The significance of this work is profound, as it has the potential to enhance the quality and safety of spices available in the market, reduce the incidence of foodborne illnesses, safeguard consumers' well-being, and ensure the integrity of the spice supply chain. Furthermore, by addressing this research gap, the study can pave the way for the development of more efficient and sustainable decontamination methods, thereby benefitting both the spice industry and public health. The entity in charge of establishing standards for matters pertaining to human health is the Codex Alimentarius Commission (Codex). The Codex Alimentarius General Principles of Food Hygiene (Awuchi, 2023) must be followed when irradiating food, along with excellent management practices. The irradiation of at least one food product is legal in about 60 nations worldwide (Mshelia *et al*., 2023). According to the Joint FAO/IAEA/WHO Expert Committee, irradiation of up to 10 kGy has no toxicological hazards and has no negative nutritional effects on food (Ravindran and Jaiswal, 2019). Fungal contamination in spices is a pervasive issue, leading to the potential presence of mycotoxins, which pose severe health risks when consumed. By investigating the application of gamma radiation as a decontamination method, this research seeks to provide a safe and practical solution for the spice industry. Not only can this study help enhance the quality and safety of spices available in the market, but it also contributes to reducing the incidence of foodborne illnesses, safeguarding consumers' well-being, and ensuring the integrity of the spice supply chain. This method will increase food safety without affecting quality while reducing post-harvest loss and extending shelf life. In Bangladesh, many spices are cultivated every year, but unfortunately, drying conditions cannot remain accurate (Rahman *et al*., 2018). Therefore, the level of fungal contamination in spices needs to be identified. To the best of the author's knowledge, this is the most important study of its kind to investigate mycotoxin's presence in Bangladesh's spices. The research had three goals: (i) to find the fungi that were in seven different kinds of spices; (ii) to use PCR to find the *A. flavus* strains that were in the spices; and (iii) to find the best gamma irradiation doses to get rid of the fungi that were in the spices.

MATERIALS AND METHODS

Sample collection and processing

In May 2021, 49 spice samples were gathered from Dhaka City's neighborhood markets in Bangladesh. Samples were ginger (*Zingiber officinale*), black pepper (*Piper nigrum*), garlic (*Allium sativum*), coriander (*Coriandrum sativum*), turmeric (*Curcuma longa*), cumin (*Cuminum cyminum*), and red chili (*Capsicum annuum*). Composite samples of spices were prepared by homogeneously mixing each type of sample for fungal analysis. Samples were analyzed as duplicates. Spices were collected in powder form and chosen based on their popularity, usage, and availability in the markets. Samples were stored at room temperature in the laboratory until analysis.

Irradiation and dosimetry

Six groups of about 20 g of each spice sample were placed into glass vials. One group of each sample was kept as a control and not exposed to gamma rays, while the other four were treated to 2, 4, 6, and 10 kGy. The experiment was conducted at the Atomic Energy Research Establishment in Dhaka, Bangladesh's Institute of Food and Irradiation Biology (IFRB). A Gamma Beam 650 from AECL in Canada was used to irradiate the samples. Cobalt-60 was used as the radiation source. The 50 kCi gamma irradiator's activity increased when the spices were exposed to it. According to the accepted approach (Rahman *et al*., 2021) ferrous sulfate (Fricke) and red perspex dosemeters were used to measure the absorbed dose. Standardized against Fricke dosimeters were red perspex dosemeters (Type 4034 AF, AERE, Harwell, UK; dose range 5-50 kGy).

Moisture content and pH

To calculate the samples' moisture content, powdered spices

were properly combined. By using an automatic moisture analyzer (Kern and Sohn GmbH, D-72336, Germany) for two hours, one (1) g of each sample was heated at 105°C (Gryczka *et al*., 2020). Spices: Distilled water=1: 9 suspensions for each sample were made and agitated for 2 hours in a 200 ml beaker in order to obtain the pH values (Rahman *et al*., 2021). Using a digital pH meter from Edge®, the pH of the remaining samples was calculated.

Calculation of radiation D-values

In this study, D_{10} values were computed in accordance with Fan *et al*., 2023. Here, N_0 is the beginning microbial content (cfu/ml), D is the radiation dose (kGy), and N is the number of bacteria that survive after irradiation (cfu/ml). The D_{10} value, which is −1/b, shows how much radiation is needed to kill 90% of microorganisms. Here, b is the slope of the logarithmic function of the radiation dose against the number of living bacteria. Finally, this equation is used for the calculation of D values: $D= D_{10} \times \log 10(N_0/N)$.

Mycological analysis

About 10 g of each homogenized spice sample was transferred into a screw-capped bottle with 90 ml of sterile distilled water and mixed properly (Kumar *et al*., 2020). Serial dilutions of each sample were plated onto sterile plastic Petri dishes containing solidified potato dextrose agar (PDA) (Liofilchem, Italy) and incubated at the inverted position at 28°C for 3 days (Khalid *et al*., 2018). After the samples were left to grow, the total number of fungi found per gram was found by multiplying the average number of colonies per plate by the dilution. After spore isolation on PDA, fungal cultures were preserved in the refrigerator at -4 ° C until analysis.

Isolation of *Aspergillus flavus*

A. flavus was isolated thanks to the microscopy examination. Each distinct colony's macro- and microscopic structures were employed. A loop of pure isolates that had been grown on PDA was then grown on malt extract agar (MEA) (Oxoid Ltd., Hampshire, UK) to help with identification. The MEA plates were incubated for 7 days at 25°C. Gross appearance and microscopic characteristics were used to identify fungi (Gnonlonfin *et al*., 2013).

Fungal DNA isolation extraction

This research put 100 mg of scratched mycelium from different fungi into a microcentrifuge tube (2.0 ml Eppendorf tube), quickly frozen it in liquid nitrogen, and then crushed it very well. Pulverized mycelia were added to 1300 microliters of lysis buffer (100 mM Tris HCL [pH 8.0], 50 mM EDTA, 3% SDS), which were then incubated at 65 °C for 30 minutes. The technique outlined by Bansal *et al*. 2019 was used to extract the fungal genomic DNA. In 30 microliters of TE buffer, the isolated DNA pellet was dissolved.

Fungal identification by ITS1-ITS4

This study used the universal primers ITS1 and ITS4 to sequence the 5.8S ITS rRNA and DNA nucleotides (600 bp) of fungal samples (Tahir *et al*., 2020). 12.5 µl of Master Mix (Promega, USA), 0.2 µl of Tag (Promega, USA), 2 µl of forward and reverse primers, 2 µl of DNA templates, and 8.3 of ultrapure water were used in the PCR process. The thermocycler was programmed to perform the following steps: initial denaturation at 94 °C for 5 min; 35 cycles of 30 s at 94 °C for denaturation, 30 s at 54 °C for annealing, and 30 s at 72 °C for elongation; and finally, a final elongation step of 5 min at 72 °C. 1% agarose gel electrophoresis was used to examine the PCR results, and they were then stained with a 0.5 mg/ml ethidium bromide solution and examined under UV light.

PCR purification and gene sequencing

The Pure LinkTM PCR Purification Kit (USA) was used to clean the PCR products, and the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) was used to sequence them on a 3130 genetic analyzer.

Aflatoxin-producing gene identification

DNA from *A. flavus* was taken out, and PCR was used to make more copies of the *aflP*, *aflR*, and *aflM* gene markers. The 25 µl PCR reaction mixture had 12.5 µl of master mixes, 2 µl of forward and reverse primers, 2 µl of DNA templates, 8.2 µl of ultrapure water, and 0.3 µl of Taq DNA polymerase (Promega, USA). The PCR settings were as follows: a 5-minute initial denaturation step at 94 °C, then 30 cycles of 1 minute at 94 °C for denaturation, 2 minutes at 65 °C for annealing, 2 minutes at 72 °C for the extension, and a final 5-minute extension step at 72 °C. On a 1% agarose gel with ethidium bromide solution (0.5 mg/ml) added, the PCR products were examined and then seen in UV light (Hammami *et al*., 2014).

Statistical analysis

At least three separate analyses were performed for each. Standard deviation and mean values were used to express the results. Utilizing Statistical Software R (Windows version 3.3.2), statistical analysis was carried out. The significance of the difference between the mean values at the level of $p \leq 0.05$ was assessed using Duncan's test.

RESULTS AND DISCUSSION

Fungal mycoflora in non-irradiated and irradiated spice samples

In this study, Table 1 shows the total number of fungi found in spice that were irradiated and samples that were not. Mean fungal counts were found in spices following the descending order of red chili > turmeric > black pepper > coriander > garlic > cumin > ginger. In this research, total fungal counts in non-irradiated spice samples were found to range from 1.87×10^3 to 8.50×10^4 cfu/g. The presence of a fungal load was detected in red chili powder at 8.50×10^4 cfu/g, and after 180 days, the fungal load was found to be 1.08×10^5 cfu/g. Similarly, the rate of contamination increased rapidly for all non-irradiated spices during the 180-day storage period. Similar investigations found

Table 1. Effects of gamma radiation doses (kGy) on fungus load in spices during 180 days storage.

Doses	Storage (days)	Red chili	Turmeric	Cumin	Coriander	Garlic	Ginger	Black pepper
0	0	8.50×10^4	4.23×10^4	2.93×10^3	4.80×10^3	4.77×10^3	1.87×10^3	3.10×10^4
	180	1.08×10^5	9.66×10^4	6.42×10^3	8.22×10^4	8.47×10^3	4.92×10^3	7.66×10^4
2	0	2.60×10^3	3.10×10^2	ND	ND	ND	ND	2.16×10^2
	180	2.40×10^3	2.24×10^2	ND	ND	ND	ND	1.28×10^2
4	0	2.06×10^2	-	-	-	-	-	-
	180	2.06×10^2	-	-	-	-	-	-
6	0	-	-	-	-	-	-	-
	180	-	-	-	-	-	-	-

ND means not detectable (detection limit 10 cfu/g); Dash (–) = Means no colony was found in samples; Values are means of triplicate experiments (n=3).

Table 2. Effects of gamma radiation doses (kGy) on physicochemical properties in spices during 180 days of storage.

Doses	Storage (days)	Red chili (n=3)	Turmeric (n=3)	Cumin (n=3)	Coriander (n=3)	Garlic (n=3)	Ginger (n=3)	Black pepper (n=3)
Moisture								
0	0	6.64 ± 0.01^{ab}	7.89 ± 0.01^{a}	7.79 ± 0.01^{a}	5.62 ± 0.02^{a}	7.53 ± 0.03^{a}	6.83 ± 0.02^{a}	7.49 ± 0.01^{b}
	180	7.57 ± 0.02^{ab}	7.98 ± 0.01^{a}	8.26 ± 0.01^{a}	6.83 ± 0.02^{a}	7.65 ± 0.02^{a}	7.25 ± 0.01^{a}	7.88 ± 0.03^{a}
2	0	6.58 ± 0.02^{b}	7.77 ± 0.01^{a}	7.80 ± 0.01^{a}	5.61 ± 0.01^{a}	7.42 ± 0.03^{a}	6.84 ± 0.02^{a}	7.51 ± 0.01^{ab}
	180	7.65 ± 0.12^{ab}	7.92 ± 0.02^{a}	8.23 ± 0.02^{a}	6.28 ± 0.01^{a}	7.68 ± 0.06^{a}	7.26 ± 0.01^{b}	7.84 ± 0.02^{a}
4	0	6.65 ± 0.01^{a}	7.82 ± 0.03^{a}	7.76 ± 0.06^{a}	5.62 ± 0.02^{a}	7.22 ± 0.03^{a}	6.83 ± 0.01^{a}	7.53 ± 0.02^{a}
	180	7.52 ± 0.01^{a}	7.92 ± 0.02^{a}	8.22 ± 0.01^{b}	6.23 ± 0.03^{a}	7.69 ± 0.01^{a}	7.21 ± 0.01^{a}	7.81 ± 0.01^{a}
6	0	6.65 ± 0.01^{a}	7.88 ± 0.01^{a}	7.75 ± 0.02^{b}	5.60 ± 0.02^{a}	7.20 ± 0.01^{a}	6.80 ± 0.02^{a}	7.53 ± 0.02^{a}
	180	7.54 ± 0.02^{b}	7.95 ± 0.01^{a}	8.09 ± 0.01^{b}	6.21 ± 0.01^{a}	7.65 ± 0.03^{b}	7.22 ± 0.01^{a}	7.85 ± 0.01^{a}
pH								
0	0	5.87 ± 0.01^{b}	6.69 ± 0.01^{ab}	6.05 ± 0.02^{a}	6.63 ± 0.01^{a}	6.06 ± 0.01^{a}	6.20 ± 0.02^{a}	6.18 ± 0.03^{a}
	180	5.72 ± 0.01^{b}	6.29 ± 0.01^{b}	5.82 ± 0.02^{a}	6.46 ± 0.02^{a}	5.82 ± 0.02^{a}	5.91 ± 0.01^{b}	5.79 ± 0.01^{a}
2	0	5.83 ± 0.01^{c}	6.68 ± 0.01^{ab}	6.08 ± 0.03^{a}	6.62 ± 0.02^{a}	6.04 ± 0.01^{a}	6.14 ± 0.01^{b}	6.19 ± 0.01^{ab}
	180	5.72 ± 0.01^{a}	6.27 ± 0.03^{a}	5.78 ± 0.03^{b}	6.44 ± 0.01^{a}	5.83 ± 0.01^{a}	588 ± 0.01^{c}	5.80 ± 0.04^{a}
4	0	5.86 ± 0.01^{c}	6.72 ± 0.01^{a}	6.06 ± 0.02^{a}	6.62 ± 0.02^{a}	6.07 ± 0.01^{a}	6.16 ± 0.04^{b}	6.12 ± 0.01^{b}
	180	6.64 ± 0.01^{ab}	7.89 ± 0.01^{a}	7.79 ± 0.01^{a}	5.62 ± 0.02^{a}	7.53 ± 0.03^{a}	6.83 ± 0.02^{a}	7.49 ± 0.01^{b}
6	0	5.82 ± 0.02^{b}	6.65 ± 0.03^{a}	6.01 ± 0.01^{a}	6.65 ± 0.01^{a}	6.00 ± 0.01^{b}	6.20 ± 0.02^{a}	6.15 ± 0.02^{a}
	180	6.55 ± 0.02^{a}	7.94 ± 0.02^{a}	7.71 ± 0.02^{b}	5.60 ± 0.01^{a}	7.52 ± 0.02^{a}	6.79 ± 0.01^{b}	7.50 ± 0.01^{a}

[a-c]Mean ±SD, mean values (n=3) with the same row with different lowercase letters are significantly different at p≤0.05 among the radiation dose.

the number of fungi present in paprika in Hungary ranged between mold ($7.22\times10^3\pm5267$ cfu/g) and yeast ($1.42\times10^3\pm1746$ cfu/g) (Molnar *et al*., 2018), in Saudi Arabia ($1.0\text{-}408\times10^3$ cfu/g) (Wikandari *et al*., 2020), in Ethiopia (4.0 and 5.4 log CFU/g yeast and mold, respectively) (Woldemariam *et al*., 2021), and in Korea (1.56-7.15 log CFU/g) (Ramalingam *et al*., 2022). Moreover, total fungal counts were found after 2 kGy gamma irradiation treated red chili (2.60×10^3 cfu/g), turmeric (3.10×10^2 cfu/g), and black pepper (2.16×10^2 cfu/g), and the fungal count was not detected in other spices. Additionally, fungal growth was reduced in irradiated spices during the 180-day storage period. In this study, no fungal growth was detected when applying 6, 8, and 10 kGy doses to spices. This investigation found 6 kGy identified as an optimum gamma radiation dose for red chili, 4 kGy identified for turmeric and black pepper, and 2 kGy identified for cumin, coriander, garlic, and ginger. Fungal contamination rates were lower in garlic and ginger samples due to antifungal activity (Hammami *et al*., 2014). A similar study reported that the optimum gamma irradiation dose was 5 kGy in spices like red chili, turmeric, coriander, and cumin powder (Sirisoontaralak *et al*., 2022). A recent study also found that a 5 kGy dose could stop the growth of fungi in spice samples (Esmaeili *et al*., 2018).

Physicochemical properties of non-irradiated and irradiated spice samples

The moisture content and hydrogen ion (pH) values of spice samples, irradiated and non-irradiated, were determined and presented in Table 2. In this investigation, the highest and lowest moisture content values were measured at 7.89% and 5.62% in non-irradiated turmeric and coriander, respectively, at the 0-day storage period; 8.26% and 6.83% were measured in cumin and coriander powder, respectively, at the 180-day storage period. In the same way, the highest and lowest pH levels were found in non-irradiated turmeric (6.69) and red chili (5.87), respectively, after 0 days of storage. The lowest and highest pH levels were found in coriander (6.46), followed by red chili, after 180 days of storage. The high humidity and high pH of spices represent favorable conditions for fungal growth (Abrar *et al*., 2023). After analyzing the results, it was observed that low radiation doses do not significantly change the physicochemical properties even after 180 days of storage. The previous study also reported that treatment by gamma radiation <10 kGy cannot change the pH value in the spice samples (Jeong *et al*., 2020); also, there were no effects on moisture content after treatment by gamma radiation in spice samples (Hassan *et al*., 2019).

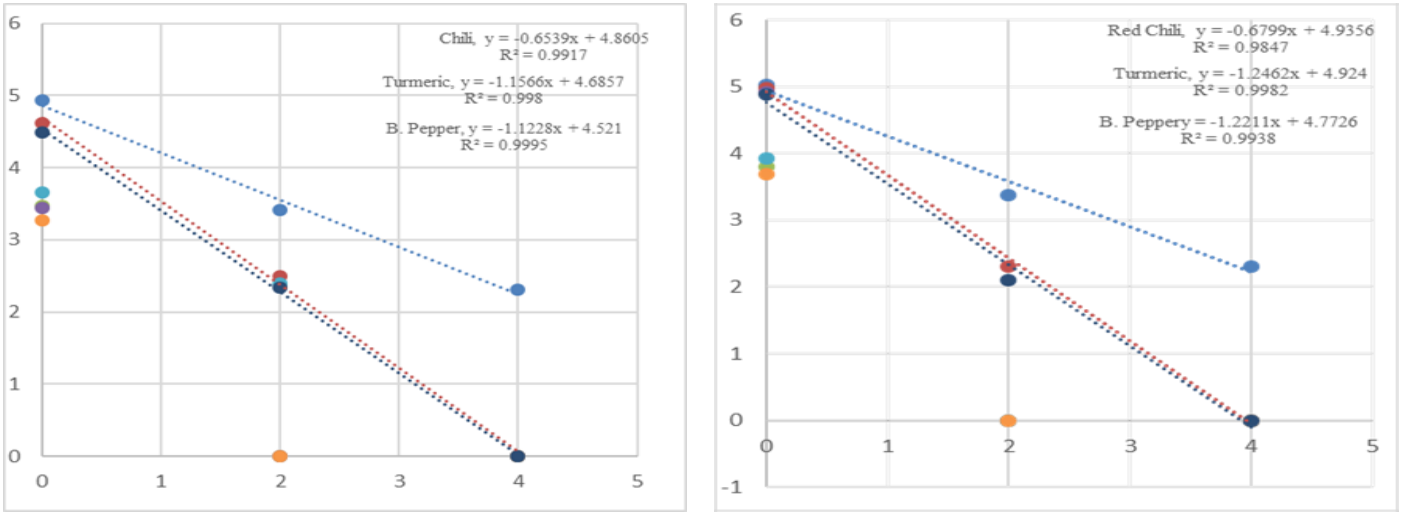
Table 3. Radiation D_{10} values (kGy) of total fungus in spices (red chili, turmeric, and black pepper) samples.

Gamma dose (kGy)	Red chili		Turmeric		Black pepper	
	0 m	6 m	0 m	6 m	0 m	6 m
D_{10} value	1.52	1.47	0.89	0.80	0.89	0.82
5D value	7.60	7.35	4.45	4.00	4.45	4.10

D10 value of cumin, ginger, garlic, and coriander cannot be calculated because fungal growth was not detectable at even 2 kGy.

Table 4. Primers used for molecular identification of fungal stains isolated from spices samples.

Gene	Primer pair	Primer sequences (5´-3´)	PCR product length (bp)	Annealing temperature (°C)	References
ITS	ITS1	TCCGTAGGTGAACCTGCGG	600	54	White *et al.* (1990)
	ITS4	TCCTCCGCTTATTGATATGC			
aflP	Omt1-F	GCCTTGCAAACACACTTTCA	1490	65	Hammami *et al.* (2014)
	Omt1-R	AGTTGTTGAACGCCCCAGT			
aflR	AflR-F	CGAGTTGTGCCAGTTCAAAA	999	65	Hammami *et al.* (2014)
	AflR-R	AATCCTCGCCCACCATACTA			
aflM	apa-F	TATCTCCCCCCGGGCATCTCCCGG	1034	65	Gnonlonfin *et al.* (2013)
	apa-R	CCGTCAGACAGCCACTGGACACGG			



Gamma dose (0 month) Gamma dose (6 months)

Figure 1. *Reduction of total aerobic bacteria in 0 and 6 months stored spices by gamma radiation.*

The previous researcher reported that the moisture content found in Bangladeshi chili powder was in the range of 5.84% (Rahman *et al.*, 2021). In another study, it was reported that the mean moisture content was 7.10% and 14.8% in garlic and pepper samples in Southern Togo (Gnonlonfin *et al.*, 2013), consistent with the findings of this study.

Radiation D_{10} values of organisms in spice samples

The D_{10} and 5D values for fungus in spice samples that have been exposed to gamma radiation are very important for keeping food safe and fresh. These values provide vital insights into the effectiveness of gamma radiation in reducing fungal contamination. The D_{10} value shows the dose needed to get rid of 90% of the fungi that are present. This information helps doctors decide how to use gamma radiation to get rid of pathogens. Fan *et al.* (2023) say that the 5D value, which shows a 99.99% reduction, makes sure that more fungi are killed, which extends the shelf life of spices and makes them safer. In this study, both the radiation sensitivity values of spice samples were presented in Table 3. The R_2 values from the obtained regression equations (Figure 1) ranged from 0.991 to 0.999 for 0-month storage samples. Similarly, the R_2 values from the obtained regression equations (Figure 1) ranged from 0.984 to 0.998 for 6 months of storage samples. The D_{10} and 5D values of six spices stored for 0 months were found in red chili 1.52 and 7.60 kGy; turmeric 0.89 and 4.45 kGy; and black pepper 0.89 and 4.45 kGy, respectively. In this investigation, the D_{10} and 5D values of cumin, coriander, ginger, and garlic cannot be calculated because bacterial colonies were not detectable at even 2 kGy. Similarly, the D_{10} and 5D values of six spices stored for 6 months were found in red chili (1.47 and 7.35 kGy), turmeric (0.80 and 4.0 kGy), and black pepper (0.82 and 4.10 kGy), respectively. The D_{10} values for *A. flavus* and *A. niger* were 0.9185 kGy (Fan *et al.*, 2023) and 01.22 kGy (Shankar *et al.*, 2020), after the first study. In order to ensure the safety of food, the D*10* value for fungi exposed to gamma radiation is crucial. By giving instructions on how to correctly use gamma radiation, fungal contamination can be effectively cut down (Balakrishnan *et al.*, 2022). This strategy addresses important issues in the food preservation sector by prolonging the shelf life of spices while preserving their microbiological safety.

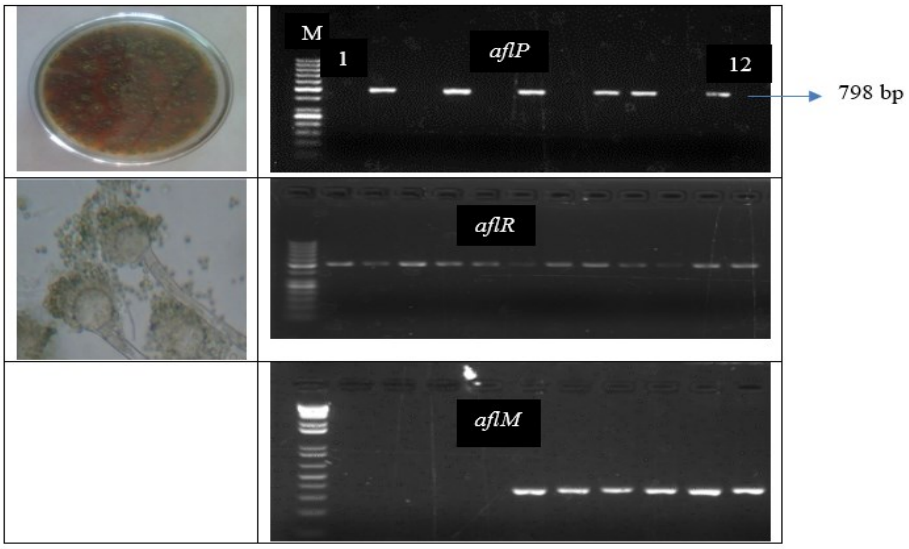


Figure 2. *Aflatoxinenic regulatory gene (aflP, aflR and aflM) identification in Aspergillus spp.*

Table 5. Aflatoxinenic regulatory gene identification of the studied spices.

Spices name	S. No.	Organisms	*aflP*	*aflR*	*aflM*
Black pepper	1	*A. flavus*	--	++	--
	2	*A. flavus*	++	++	--
	3	*A. flavus*	--	++	--
	4	*A. flavus*	++	++	--
	5	*A. flavus*	--	++	--
Red chili	1	*A. flavus*	++	++	--
	2	*A. flavus*	--	++	++
	3	*A. flavus*	++	++	++
	4	*A. flavus*	++	++	++
	5	*A. flavus*	--	++	++
	6	*A. flavus*	++	++	++
	7	*A. flavus*	--	++	++

Each (+/ −) symbol represents respectively, the presence or absence of an aflatoxigenic gene in one of the duplicate analyses.

Molecular identification of *A. flavus* in spices

Spices were collected and cultured on a PDA plate to identify the presence or absence of aflatoxigenic *Aspergillus*. In this study, a microscopic observation confirmed the presence of *Aspergillus spp*. in red chili and black pepper powder. The internal transcribed spacer (ITS) region of the fungal genome was also used to characterize *Aspergillus spp*. at the molecular level. It was possible to identify the fungus by its 5.8S ITS rDNA sequence after the fungal DNA was separated. NCBI BLAST analyzed the sequencing data and drowned it into a phylogenetic tree of closely related (99%) sequences. After phylogenetic analysis, *A. flavus* was identified in red chili and black pepper powder samples. An amplicon corresponding to 798 bp in size was seen after agarose gel electrophoresis (Figure 2). According to the results of this study, spices were contaminated with *Penicillium* (53%), *Mucor* (36%), and *Aspergillus* (16%) among the 49 spice samples. Previous studies reported that fungal contaminations were checked by traditional approaches to identify their activity (Liu *et al*., 2020; Khalid, *et al*., 2018), but molecular identification techniques are widely used to clearly characterize fungal species. Several studies have outlined that *A. flavus* contaminates spices due to improper harvesting, processing, transportation, and storage (Costa *et al*., 2019; Hammami *et al*., 2014). Moreover, *A. flavus* is the leading producer of carcinogenic aflatoxins in spice samples (Khalid *et al*., 2018).

Aflatoxin-producing gene identification by PCR

Aflatoxins are considered toxic mycotoxins for humans and animals. In a polymerase chain reaction (PCR), three aflatoxigenic primers were used to find the aflatoxin gene in *A. flavus* (Table 4). The product lengths of genes *aflP*, *aflR*, and *aflM* can be seen at 1490, 999, and 1034 bp, respectively. Based on the review of the literature, these genes were found in the spice samples, and PCR was used to check for aflatoxin contamination (Hammami *et al*., 2014). The numbers of *A. flavus* were 5 in black pepper and 7 in red chili, randomly selected to check aflatoxin genes. The results showed that 46.6% of aflatoxin genes were found in the black pepper sample and 80.95% in the red chili sample (Table 5). The previous researcher reported that aflatoxins contaminated spices, but the conventional cooking process is challenging to detoxify and does not reduce the amount of aflatoxin contamination that is already produced (Garcia *et al*., 2018). Additionally, gamma radiation is an effective technique to minimize aflatoxin contamination in spices (Mukhtar *et al*., 2023). Furthermore, the fungus is also sensitive to gamma radiation (Hosseini *et al*., 2023) because the radiation may damage DNA and cause mutations through ionization.

Conclusion

In conclusion, our study demonstrates that gamma radiation is a highly effective method for reducing fungal contamination in commercially available spices. The results indicate a significant reduction in fungal load across various spice types, highlighting its potential to enhance food safety and quality in the spice industry. In comparison to samples of cumin, coriander, garlic, and ginger, the evaluated spice samples with the highest presence of fungal contamination were black pepper powder, chili powder, and turmeric powder. In this study, the optimum gamma radiation dose was identified as 6 kGy for red chili, 4 kGy for turmeric and black pepper, and 2 kGy for cumin, coriander, garlic, and ginger. Samples of red chili and black pepper had *A. flavus* in them. The physicochemical properties of low-radiation spices (< 10 kGy) did not change before or after gamma radiation. Furthermore, gamma radiation decontamination is an environmentally friendly, non-chemical solution that ensures the safety and integrity of the spice supply chain, benefiting both consumers and the industry.

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Declaration of interest

The authors report no conflicts of interest.

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