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Research Article

Mitigation of *Aspergillus flavus* and its aflatoxins in wheat grains by gamma irradiation and calcium oxide

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Abstract: *Aspergillus flavus* is one of the important species of molds that can produce toxins during improper storage of wheat grains. In this study, different amounts of calcium oxide (0, 0.5, and 1%) were mixed with wheat samples containing mold spores. After 20 days, the samples were exposed to gamma radiation (0, 5, 10, 15, and 20 KGy). The presence of *A. flavus,* Aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2) was assessed in samples. The results indicated that the effects of calcium oxide, gamma irradiation, and their interactions were significant on *A. flavus,* AFB_1, and AFB_2 contamination. Furthermore, other toxins like AFG_1 and AFG_2 were not found in the samples. An additional reduction in AFB_1 and AFB_2 was observed when irradiation was accompanied by Cao, and the maximum inhibition of aflatoxin production was achieved at 0.5% CaO. Consequently, based on the standard maximum limit of 10 KGy for cereals, the findings of this research suggest that 0.5% of calcium oxide and 10 KGy of irradiation could be applied in the storage of wheat grains to mitigate *A. flavus*, AFB_1, and AFB_2.

Keywords: *Aspergillus flavus*, aflatoxins, wheat grains, gamma radiation, calcium oxide

Introduction

Wheat has been considered as one of the most important sources of food for human consumption, which is rapidly infected with *Aspergillus flavus* due to poorly maintained storage conditions. The growth of this fungus causes the production of aflatoxins such as B_1, B_2, G_1, and G_2, in addition to food degradation and wheat spoilage (Whitaker, 2003). Furthermore, the presence of these toxins in flour and the products thereof and their consumption can cause acute liver injury, liver cirrhosis, tumor induction, and teratogenic and carcinogenic effects (IARC, 2012). Thus, to reduce or eliminate the *A. flavus* and its toxins, several methods have been studied. Some methods dealt with the use of chemicals such as ozone (Savi *et al.*, 2015), calcium hydroxide (Elias-Orozco *et al.*, 2002), sodium bicarbonate and potassium carbonate (Amézqueta *et al.*, 2008), CO_2 (Mahbobinejhad *et al.*, 2019) and their effectiveness in the elimination of aflatoxins and other mycotoxins were evaluated. New techniques such as microwave heating (Kaur *et al.*, 2014), UV-C radiation (Ghanghro *et al.*, 2016), and pulsed electric field (Vijayalakshmi *et al.*, 2018) was also used, which caused the destruction of aflatoxin in wheat seeds. Furthermore, gamma radiation is known as a method of control and reduction of fungal toxin

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production. In one research, the 5 KGy gamma-ray irradiation process in wheat resulted in a considerable decrease in AFB_1 and was accompanied by a reduction in protein and an increase in carbohydrates (Aziz *et al.*, 2004).

It was also reported that irradiation doses (5-20 KGy) and wheat moisture levels (9-17%), did not cause significant effects on the reduction in AFB_1 concentration, whereas irradiation caused a decrease in the T-2 toxin (Hooshmand *et al.*, 1995). The complete elimination or decomposition of aflatoxins requires a high radiation dose (due to their resistance to gamma radiation), the high-level radiation causes the decomposition of various food components in cereal grains (Siddhuraju *et al.*, 2002) therefore, it is recommended that alternative methods or materials be provided to increase the efficacy of irradiation for food decontamination and shelf-life extension (Pankaj *et al.*, 2018). For this purpose, some attempts have been carried out. In one research, the combined effect of potassium sorbate and irradiation on the maintenance of strawberry quality was assessed (Al-Kuraieef *et al.*, 2019) and in another study, the interaction effect of radiation treatment and modified atmosphere packaging (MAP)on the shelf-life of fresh figs was reported (Waghmare *et al.*, 2018).

Calcium oxide is considered one of the important compounds in the food industry that is mainly used for purification processes. This material reacts with water to produce calcium hydroxide, which is used through heating to treat and soften the corn pericarp in countries like Mexico (known as the nixtamalization process). Also, a study has shown that calcium oxide significantly reduces mycotoxins (Schaarschmidt *et al.*, 2019).

However, despite separate studies on the effect of calcium oxide and radiation on the decomposition of fungal toxins in cereals, there has been no report on the simultaneous influence of calcium oxide and radiation in cereals to reduce the growth of *A. flavus* mold and its toxins production. Therefore, the purpose of this study was to assess the suitable gamma dose as well as to identify the effect of gamma irradiation and calcium oxide on *A. flavus* population and aflatoxins concentration.

Materials and Methods

Wheat seed (cultivar Number N-87-20, Golestan Agricultural and Natural Resources Research and Education Center, Iran), *A. flavus* (code: PTCC5004, Pasteur Institute of Iran), culture medium (Sabouraud 4% dextrose agar, Merck, Germany), calcium oxide (Calcium oxide powder; assay: 95%; SRL Co, India).

Sample preparation

Wheat grains without apparent physical damage, insect infestation, and aflatoxin contamination (confirmed by the Golestan Agricultural and Natural Resources Research and Education Center, Gorgan, Iran) were selected and transferred to the laboratory in sterile plastic bags. The specimens were disinfected by immersion in 5% H_2O_2 solution for 5 minutes and washed 3 times with sterile distilled water. Following this, the moisture content of the samples was adjusted to 25% (w/w).

Preparation of the fungal suspension for inoculation

The purchased strain of *A. flavus* was restored in Sabouraud Dextrose Agar medium (sterilized by Autoclave, model; DS8000255, Behdad, Iran), to form a conidial suspension of the mold. To impregnate the samples with fungal colonies of *A. flavus*, their 7-day colonies were employed. For harvesting, the fungal colonies, 5 ml of normal sterile saline was poured into the plates and mixed for 15 min at 100 rpm in the rotator shaker (VDRL, Bazianlab, Iran). Using Hemocytometric laminae, 10^6 fungal spores per ml were prepared for inoculum. Then, for sample inoculation, each of 200 g wheat (weighted by scale, model; TE15025, Sartorius, USA) was sprayed with 2 ml fungal suspension. The samples were then incubated for 20 days at 25 °C (using a refrigerator incubator model; aqualytic Faks-1802, Germany) with a relative humidity of 97-98% (Refai *et al.*, 1996). Before incubation, the

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contaminated wheat was uniformly sprayed with CaO powder to the determined concentrations of 0.5% and 1% (w/w).

Samples radiation

The inoculated wheat grains were exposed to different doses of 0, 5, 10, 15, and 20 KGy, emitted by the Cobalt-60 (Dose rate 4.9 Gy/min, gamma cell model; Issledovapel-Px30, Russia) at Iran's Atomic Energy Organization, Nuclear Agriculture Research Institute, Karaj, Iran.

Enumeration of *A. flavus* colonies

Aspergillus flavus colony counts were performed by standard reference methods (ISO, 2008). For the detection of aflatoxins in the samples, 25 g of each sample was ground with a powder mixer, then extracted with methanol containing 4% KCl. The extracts were purified with a 30% ammonium solution and the extraction of aflatoxins was undertaken with the addition of chloroform. The toxins were identified and determined using high-performance liquid chromatography (Waters E2695). The detector was a multi-wavelength fluorescence detector (Waters 2475) and was used in the following conditions: excitation of 362 nm, emission of 426 nm for aflatoxins B_1 andB_2, and emission of 465 nm for aflatoxins $G_1$and G_2. The samples were analyzed at the H_2O/ACN/MeOH (6:2:3 v/v/v) mobile phase, 60-µl injection volume, and a flow rate of 2 ml/min and 40 °C using a 100 mm × 4.6 mm id Chromolith analytical column (Phenomenex Inc., USA) (16050 ISO, 2003).

Statistical analysis

A full factorial designed experiment was conducted to examine the calcium oxide and gamma irradiation on the control of aflatoxins. The factorial design consisted of all possible combinations of levels for all factors. In this experiment the first factor was calcium oxide consisted of three-level (0, 0.5, and 1%) and gamma irradiation at five levels (0, 5, 10, 15, and 20 KGy). When factors were significantly different, the treatments were grouped by Tukey's test at $P < 0.05$. The statistical analysis was performed by SPSS version 16.0 The Generalized Linear Model was conducted by the response surface method (Design-Expert V. 10) to describe the distinctive and interactive effects of calcium oxide and gamma irradiation on *A. flavus*, AFB_1, AFB_2, AFG_1, and AFG_2.

Results

Effect of calcium oxide and irradiation on *A. flavus* population

In this study, the addition of calcium oxide has a significant effect on the amount of *A. flavus* ($F = 20.098$; $df_v = 2$; $df_e = 30$; $p < 0.01$). This effect is dependent upon the quantity of calcium oxide. For example, in non-irradiated samples containing 0.5% and 1% calcium oxide, *A. flavus* increased by 39.9% and 12.73%, respectively (compared to the control). In the irradiation analysis, its negative effect on the population of *A. flavus* ($F = 548.77$; $df_v = 4$; $df_e = 30$; $p < 0.001$) was determined, as the population was reduced by increasing the intensity of the irradiation, so that the highest reduction (84 to 85%) was recognized at 20 KGy (Table 1). The analysis of variance also showed that in the mildew population, there was a significant interaction between calcium oxide and irradiation ($F = 7.74$; $df_v = 8$; $df_e = 30$; $p < 0.001$). As shown in Table 1, fluctuations in the population of mold are notable, as they decrease by increasing the intensity of irradiation, but a change occurred in the presence of calcium oxide. While the average reduction in the mold in the irradiation process was 49.07%, this loss in the presence of 0.5% and 1% calcium oxide was 41.15% and 43.7%, respectively (Table 1). Concerning fungal loading, the non-irradiated sample containing 0.5% calcium oxide (No. 6) revealed the maximum presence of *A. flavus* (509333 cfu/g), but the minimum values were detected in samples 5, 10, and 15.

Depending on the fungal population, the treatments were categorized into five groups. The largest group comprised seven samples (No. 3, 4, 5, 9, 10, 14, and 15), and the smallest group consisted of a single treatment (No. 6). Table 1 shows the mean *A. flavus* values and the similarities or differences between treatments.

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Table 1 Means and standard deviation values of *A. flavus* population, Aflatoxin B_1 and B_2 concentrations in wheat.

Samples	CaO (%)	Irradiation dose (KGy)	*A. flavus* (Cfu × 10^3/g)[1]	*A. flavus* Reduction (%)	AFB_1 (ppb)[1]	AFB_1Loss (%)	AFB_2 (ppb)[1]	AFB_2Loss (%)
1	0	0	366.0 ± 25.1^{b}	0	8802 ± 58^{b}	0	1241 ± 6^{a}	0
2	0	5	252.0 ± 22.1^{c}	31.06	4624 ± 57^{e}	47.47	1033 ± 15^{b}	16.71
3	0	10	125.0 ± 18.0^{de}	65.83	4180 ± 72^{f}	52.51	936 ± 9^{c}	24.56
4	0	15	93.3 ± 15.3^{e}	74.28	3757 ± 81^{g}	57.32	833 ± 12^{d}	32.86
5	0	20	53.3 ± 10.4^{ef}	85.36	3257 ± 51^{i}	63.00	672 ± 8^{e}	45.86
Average (sample 1-5)				49.07		44.06		30.00
6	0.5	0	509.3 ± 41.0^{a}	-39.90	6307 ± 31^{c}	28.34	220 ± 15^{h}	82.27
7	0.5	5	293.3 ± 25.2^{c}	19.91	3407 ± 38^{h}	61.30	170 ± 6^{i}	86.32
8	0.5	10	159.7 ± 20.0^{d}	56.49	2287 ± 61^{j}	74.02	130 ± 5^{j}	89.52
9	0.5	15	97.3 ± 12.5^{e}	73.48	1877 ± 21^{k}	78.68	112 ± 7^{jk}	90.97
10	0.5	20	55.3 ± 15.0^{ef}	84.65	1573 ± 25^{l}	82.12	87 ± 4^{k}	93.01
Average (sample 6-10)				41.15		64.89		88.42
11	1	0	411.7 ± 17.6^{b}	-12.73	9233 ± 21^{a}	-4.91	462 ± 20^{f}	62.78
12	1	5	304.3 ± 19.1^{c}	16.62	5637 ± 42^{d}	35.96	254 ± 11^{g}	79.50
13	1	10	162.0 ± 8.2^{d}	55.64	4730 ± 46^{e}	46.26	180 ± 8^{i}	85.52
14	1	15	92.7 ± 12.5^{e}	74.76	4190 ± 26^{f}	52.39	125 ± 11^{j}	89.89
15	1	20	57.3 ± 2.5^{ef}	84.25	3673 ± 31^{g}	58.27	90 ± 6^{k}	92.77
Average (sample 11-15)				43.71		37.6		82.10
Average (sample 6-15)				42.43		51.24		85.25
Total average (sample 1-15)				44.64		48.85		64.83
F			164		6599		4321	
df_v			14		14		14	
df_e			30		30		30	
P			< 0.0001		< 0.0001		< 0.0001	

[1] Values are means of three replicates ± standard deviation (SD). Values followed by the same letter are not significantly different ($P < 0.05$) according to the Tukey's test.
% Loss was calculated based on the control sample (0% CaO, 0 KGy).

Effect of calcium oxide and irradiation on AFB_1

Based on the results of the variance analysis of the data, calcium oxide had a significant effect on the creation of AFB_1, leading to both a decrease and increase in AFB_1 ($F = 10395.5$; $dfv = 2$; $dfe = 30$; $p < 0.001$). As seen in Table 1, the concentration of AFB_1 was reduced by 28.34% in the non-irradiated sample containing 0.5% calcium oxide, but at 1% calcium oxide the AFB_1content increased by 4.91%. In the non-irradiated samples containing 0.5% calcium oxide, the lowest concentration of AFB_1 was observed despite the highest *A. flavus* populations. However, at the 1% concentration of calcium oxide, despite the decline in the *A. flavus* mold population, the concentration of AFB_1 increased by about 4.91% (compared to the control). Similar to the calcium oxide results, the irradiation also had a significant effect on the AFB_1 values ($F = 17733.35$; $df_v = 4$; $df_e = 30$; $p < 0.001$), so that the AFB_1 content decreased with increased irradiation. In this research, there was a significant interaction ($F= 82.94$; $df_v = 8$; $df_e = 30$; $p < 0.001$) between calcium oxide and irradiation at AFB_1 values. AS shown in Table 1, in the 0.5% calcium oxide concentration and 20 KGy intensity, the highest AFB_1 loss or lowest AFB_1 concentration was detected. While the maximum destructive effect of irradiation on AFB_1 was 63%, this loss reached the highest value at 0.5% calcium oxide (i.e., 82%). However, the preventative role of 1% calcium oxide in the production of $AFB_1$was not successful. Among the treatments, non-irradiated wheat-containing 1% calcium oxide (No. 11) was dominant in AFB_1 (6307 ppb).

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While in the sample containing 0.5% calcium oxide and 20 KGy radiation (No. 10), the lowest value of AFB_1 with a drop of 82% was detected. Table 1 shows average AFB_1 values that have been separated into 12 groups.

Effect of calcium oxide and irradiation on AFB_2

The variance analysis of the obtained data has demonstrated the effective role ($F = 26429.37$; $df_v = 2$; $df_e = 30$; $p < 0.001$) of CaO in the loss of AFB_2. As shown in Table 1, there are clear changes in the AFB_2.It was also determined that the radiation had a significant effect on AFB_2 ($F = 1524.37$; $df_v = 4$; $df_e = 30$; $p < 0.001$), such that the AFB_2 values were reduced by the increase in the radiation dose.

In the calcium-free group, the reduction in AFB_2 (16.71-45.86%) was lower than in groups containing 0.5 and 1% Cao. The role of calcium oxide in decreasing radiation intensity has been demonstrated by the analysis of the variance of the data. Table 1 clearly shows that in the presence of CaO, there is an increase in AFB_2 destruction. Even more, destruction was observed concomitantly with an increase in the irradiation dose. In this test, the common role of calcium oxide and irradiation in AFB_2 concentration was very significant ($F = 193.09$; $df_v = 8$; $df_e = 30$; $p < 0.001$). However, the role of calcium oxide was more effective than irradiation. While the average destructive effect of irradiation in AFB_2 was only 30%, this effect at 0.5% and 1% CaO reached the maximum value of 88.42% and 82.1%, respectively (Table 1). As shown in Table 1, depending on the reduction in AFB_2 levels, some of the samples treated with 0.5% and 1% calcium oxide were similar to each other. In the AFB_2 assay, the maximum AFB_2value was detected in sample No. 1 (0% calcium oxide and 0 KGy), and the minimum value of AFB_2 was found in 0.5% calcium oxide and 20 KGy (sample No. 10). According to Tukey's test, treatments No. 8, 9, and 14 were in the same group and treatments No. 9, 10, and 15 were in another group (Table 1). Compared to the blank treatment, 93% of AFB_2 was eliminated in samples No. 10, and 15.

The average amount of degradation of AFB_2 in the presence of various amounts of calcium oxide and irradiation was 85.25%, whereas for AFB_1 and *A. flavus* it was 51.24% and 42.43%, respectively (Table 1). Results demonstrate the greater sensitivity of AFB_2 to calcium oxide. Table 1 shows the analysis of the AFB_2 value and similarities or differences between treatments according to Tukey's tests.

Detection of AFG1 and AFG2

In this assay, AFG_1 and AFG_2 toxins were not identified in any of the treatments.

Discussion

In this study, it was found that the addition of calcium oxide has miscellaneous effects on the amount of *A. flavus*. These effects vary depending on the amount of calcium oxide since optimal CaO values have led to increased mold growth, due to the role of calcium in improving metabolism and calcium supply (Viquez *et al.*, 1994). But at high calcium oxide concentration, the reduction in *A. flavus* population is associated with an increase in the concentration of calcium ions in cytocells, which is toxic to fungi. Compared to the present study, a much lower concentration of calcium (300 ppm calcium chloride) was reported as a growth prevention factor for *Botrytis cinerea* (Boumaaza *et al.*, 2015).

In the radiation assessment, *A. flavus* population was decreased with an increase in radiation intensity. Different studies revealed various findings based on the surviving population and radiation dose level in food samples. In Aquino's study, *A. flavus* sensitivity was much higher than in our research, where a reduction of more than 99% was seen in the 10 Kgy irradiated corn mold population (initial value of 6×10^6cfu/g) (Aquino *et al.*, 2005).In another study, half of the 5 KGy gamma radiation prevented *A. flavus* sporulation, germination, and growth in corn and feed samples (Markov *et al.*, 2015). Based on one study, the amount of irradiation to destroy total *Aspergillus fungi* in lotus seeds

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(with an initial count of 50,000 cfu/g) was found to be 10 KGy (Bhat *et al.*, 2010). In Khorasani's study noted the complete mortality of *A. flavus* spores with 5 KGy gamma rays in pistachio samples (Khorasani *et al.*, 2018). It seems that other factors such as the initial mold population, the water activity (aw) or the relative availability of water in a substance and percentage of the culture medium constituents (Ghanem *et al.*, 2008), and compounds produced by gamma ray (Aquino, 2011) are responsible for the gamma-ray resistance of *A. flavus*.

In our study, it was found that the mold populations in wheat samples could also be affected by irradiation and calcium oxide. Calcium oxide caused not only an increase but a decrease in AFB_1 as well. So that at a higher concentration of calcium oxide, the concentration of AFB_1 increased, which was accompanied by a decline in the *A. flavus* population. However, at the lower calcium oxide content, the concentration of AFB_1 declined, especially in the non-irradiated sample. It appears that the 1% increase in calcium oxide concentration leads to a change in mold growth from the logarithmic to the stationary phase. This results in a reduced mold population and the production of certain secondary metabolites such as AFB_1 (Jay *et al.*, 2005). Jayashree *et al.* (2000) have reported the importance of calcium-calmodulin (Ca^{2+}-Calmodulin) and its role in the phosphorylation/dephosphorylation ratio for aflatoxin production using *Aspergillus parasiticus* (Jayashree *et al.*, 2000). Similar to our finding, a 40% reduction in AFB_1 was reported in 2% calcium oxide-treated corn grains during the heating process. The increased calcium oxide concentration did not significantly reduce the toxin and even led to undesirable flavor changes in the product (Abbas *et al.*, 1988). Moreno-Pedraza applied 1% calcium oxide in addition to 90 °C heating in corn processing (tortilla production). Compared with the initial concentration of 125 ppb, a 100% reduction in AFB_1 was revealed (Moreno-Pedraza *et al.*, 2015).

In this research, The decomposition of aflatoxin by gamma rays results from the indirect effects of water-released radicals or other radionuclides, which merely attack AFB_1 and reduce its bioactivity at the terminal furan ring (Rustom, 1997). Similar to the findings of this study, the reduction of AFB_1 (with an initial concentration of 37.61 ppb) in wheat grains with an irradiation dose of 8 KGy was approximately 69.3% (Mohamed-Neeven *et al.*, 2015). However, in barley seed, a greater decrease (90%) in AFB_1 (with an initial concentration of 6410 ppb) was detected at a gamma-ray level of 10 KGy (Ghanem *et al.*, 2008). It is noteworthy that the average loss of AFB_1 in our study (with an initial concentration of 8802 ppb) at 5 and 10 KGy was 48.2% and 57.6%, respectively (data not shown), which is close to Markov's findings. In that research, AFB_1 destruction (with an initial concentration of 50 ppb) in feed and corn samples at 5 and 10 KGy was 60% and 85%, respectively (Markov *et al.*, 2015). In another study, 10 KGy gamma rays caused complete degradation of AFB_1 (with a preliminary content of 2597 ppb) in corn grains (Aquino *et al.*, 2005). The reason for the different resistance of AFB_1, even in similar samples, may be linked to the initial toxin concentration, the moisture content, the fat and protein content, and the variety of products. In a study, during radiation, because of free radical formation, degradation of AFB_1, and their interactions, five compounds were produced (Liu *et al.*, 2016).

In our findings, CaO has been shown to influence AFB_2 loss. But controversial papers have been published on the role of calcium in the production of aflatoxins. Although the insignificant role of calcium in aflatoxin production has been reported (Rao *et al.*, 1999), Maggon found that calcium deficiency caused a reduction in aflatoxin production (Maggon *et al.*, 1977). The reason for these differences can be related to the type of mold, the culture medium, and growth conditions (Jay *et al.*, 2005). Based on our results, it was also determined that by increasing the irradiation rate, a decrease in AFB_2 values occurred which were lower than

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Aquino's results (Aquino *et al.*, 2005) where the intensity of 10 KGy resulted in the complete degradation of AFB_2 (with an initial concentration of 571.1ppb) in corn specimens.

In this research, the role of calcium oxide in reducing the applied radiation intensity is important, as there has been a marked increase in the destruction of AFB_2 in the presence of CaO at a constant radiation dose. There was even more destruction along with an increase in radiation intensity. It seems that the role of calcium in increasing water activity has increased the effect of gamma radiation in AFB_2. Aquino *et al.* (2005) reported an increase in aw that led to an increase in the rate of degradation of AFB_2 toxins by gamma rays. In another study, the 2% concentration of calcium oxide (calcium hydroxide solution) and heat treatment in maize processing resulted in a 28% loss of AFB_2 (Abbas *et al.*, 1988). The results of this study showed that, despite a significant reduction of the AFB_1 and AFB_2 by calcium oxide and radiation, due to the high initial concentration of toxins, the remaining toxins were much higher than the European Union's standard (maximum limits for AFB_1 and the total aflatoxins 2 ppb and 4 ppb respectively) (EU, 2006).

Conclusion

Generally, irradiation inhibited the growth of *A. flavus*, but no synergistic effect between irradiation and calcium oxide was observed. Calcium oxide led to a remarkable inhibition of aflatoxin production at 0.5%, which had a more inhibitory effect on the production of AFB_1 compared to AFB_2. Unlike the variable effects of calcium oxide concentrations, the effect of irradiation on reduction of the mold population was rather pronounced, so that increasing the dose intensity resulted in a reduction of *A. flavus* colonies and degradation of the majority of AFB_1 and AFB_2 toxins. In conclusion, based on the standard radiation dose limit of 10 KGy for cereals, it is recommended that wheat grains be treated with 0.5% calcium oxide before storage and 10 KGy after storage, in combination, to achieve the maximum inhibitory effect on *A. flavus* and its aflatoxins.

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کاهش *Aspergillus flavus* و غلظت سموم آفلاتوکسین‌های آن در دانه‌های گندم توسط اکسیدکلسیم و پرتودهی با اشعه گاما

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چکیده: *Aspergillus flavus* یکی از گونه‌های مهم قارچ‌ها است که می‌تواند سمومی را در زمان انبارداری نامناسب دانه‌های گندم تولید نماید. در این مطالعه مقادیر متفاوت اکسیدکلسیم (صفر، ۰/۵ و ۱ درصد) با نمونه‌های گندم حاوی اسپورهای *A. flavus* مخلوط شد. پس از ۲۰ روز، نمونه‌ها در معرض مقادیر متفاوت پرتودهی گاما (صفر، ۵، ۱۰، ۱۵ و ۲۰ کیلوگری) قرار گرفتند. حضور *A. flavus*، آفلاتوکسین B_1 (AFB_1)، آفلاتوکسین B_2 (AFB_2)، آفلاتوکسین G_1 (AFG_1) و آفلاتوکسین G_2 (AFG_2) در نمونه‌ها مورد ارزیابی قرار گرفت. نتایج بیانگر اثرات معنی‌دار اکسیدکلسیم، پرتودهی و برهم‌کنش آن‌ها بر *A. flavus*، سم AFB_1 و سم AFB_2 بود. در این پژوهش، سموم دیگری از قبیل AFG_1 و AFG_2 در نمونه‌ها شناسایی نشد. کاهش بیش‌تر سموم AFB_1 و AFB_2 طی همراهی پرتودهی و اکسیدکلسیم ایجاد شد و بیش‌ترین ممانعت‌کنندگی در تولید آفلاتوکسین‌ها در غلظت ۰/۵٪ اکسیدکلسیم به‌دست آمد. براساس حد استاندارد حداکثر ۱۰KGy برای دانه‌های غلات، می‌توان به‌کارگیری ۰/۵ درصد اکسیدکلسیم و شدت پرتوی ۱۰KGy برای ذخیره‌سازی دانه‌های گندم جهت کاهش *A. flavus* و سموم آفلاتوکسین AFB_1 و AFB_2 را توصیه نمود.

واژگان کلیدی: *Aspergillus flavus*، آفلاتوکسین‌ها، دانه‌های گندم، پرتودهی گاما، اکسیدکلسیم

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