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EFFECTS OF GAMMA-IRRADIATION AND CHEMICAL COMPOSITION OF SOME CROP SEEDS ON AFLATOXIN B_1 PRODUCTION BY *Aspergillus flavus*.

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ABSTRACT

The effects of gamma-irradiation on chemical composition of some different crop seeds, fungal infection and aflatoxin B_1 production by *Aspergillus flavus* were investigated. *Aspergillus flavus* infected seeds behaved differently according to their principal constituents. *Aspergillus flavus* caused an increase in protein and a decrease in lipids and carbohydrate contents of wheat, soyabean and fababean seeds. Growth of *Aspergillus flavus* and production of aflatoxin B_1 was inhibited at a dose level of 5 kGy.

Aspergillus flavus utilized carbohydrates of the seeds for its growth and aflatoxin production. Crops were arranged, in a descending order according to aflatoxin produced in seeds as, wheat > soyabean > fababean. There were no changes in chemical constituents of irradiated seeds, such as protein, lipids, and carbohydrates.

Keywords: Carbohydrates, lpids, protein, gamma irradiation, food safety, grains, aflatoxins, *A. flavus*, storage, moisture.

INTRODUCTION

Growth of moulds on food and fodder during storage causes great economic losses. Also, it reduces the quality of infected grains and diminished their sprouting, colour, taste, as well as nutritional value (Neergaard 1977). Grains in commercial trade undergo various handling and storage practices depending on climatic conditions and market demands (Russell *et al.*, 1982).

Cereal grains such as wheat, rice, corn ... etc, form a large part of the diet of the worlds population particularly in the developing countries (Mahrous *et al.*, 2001). Fababean and soyabean are considered as rich sources of easily available cheap proteins and lipids which occupy a prominent position in the nutritional diet in many developing countries (Youssef *et al.*, 1995). Because of the moisture content of grains is reduced considerably before the grains are placed in storage containers, bacterial spoilage dose not occur (Beuchat, 1984). However, deterioration, as a result of mould growth particularly mycotoxigenic moulds, may occur at intermediate water activity values (0.6-0.8) in grains, and thus represents a public health hazard (Mills, 1990). Mycotoxin contamination of grains can be difficult to be predicted because of the complex interaction of different factors, such as temperature, moisture, grain type, endogenous fungal species, storage history and length of storage (Sinha and Sinha, 1991, EL-Samahy *et al.*, 1995, Hassan and Aziz 1998 and Mahrous *et al.*, 2001). There has been increasing interest in the use of ionizing radiation for killing endogenous and toxigenic microorganisms (El-Far *et al.*, 1992, Aziz *et al.*, 1997 and Rustom, 1997).

The present investigation was carried out to study the influence of *Aspergillus flavus* infection on the chemical composition of seeds and aflatoxin B_1 production and to asses the effect of gamma irradiation on *A. flavus* growth and aflatoxin B_1 production in seeds.

MATERIALS AND METHODS

Samples:

Random samples (5) of wheat, soyabean and fababean seeds were collected from several human food stores in Cairo and Giza. On receipt, samples were stored at 4°C until moulds had been isolated. Samples of about 250 g seeds per each were thoroughly mixed and 10 g were used for preparing dilutions for fungal count and isolation.

Isolation and identification of moulds:

Fungal count of non-disinfected and surface disinfected wheat, soyabean and fababean seeds was determined using potato dextrose agar (PDA) medium and malt agar (MA) medium with 7.5% NaCl. Surface-disinfection was carried out using 2% sodium hypochloride, then seeds were washed with sterile distilled water for 3 to 5 times. Samples (10 g) were analyzed by adding 90 ml of sterile 0.1% potassium phosphate buffer (pH 7.0) containing 0.1% Tween 80, stomaching for 30 s with subsequent serial dilutions in phosphate buffer into PDA and MA plates in duplicate. After incubation for 7 days at 28°C, fungal isolates were identified according to Raper and Fennell (1977), Pitt (1979) and Pitt and Hocking (1985). Fungal isolates were maintained by regular transfer into plates of PDA containing 0.1% yeast extract and 2% sodium chloride.

Source of organism: *Aspergillus flavus* that was naturally isolated from wheat seeds in this study, known as highly aflatoxin B_1 producing fungus, was used through this study.

Preparation of spore suspension:

Aspergillus flavus was grown on a potato dextrose agar (PDA-Difco) slants for 10 days at 28°C. Spores were harvested by adding sterilized Tween 80 solution (0.01%, v/v), filtering through several layers of sterilized cheesecloth, centrifuging (3000xg) washing three times with sterilized distilled water and resuspending in sterilized Tween 80 solution. The number of spores was estimated by plate count using PDA and the suspension was adjusted to contain approximately 10^8 spores / ml.

Influence of *Aspergillus flavus* infection on the chemical changes of seeds and aflatoxin B_1 production:

Samples (200g) of wheat, soyabean and fababean seeds with an intial moisture content of 8.6, 7.8, 6.7%, respectively were elevated to 25% (wet weight basis) by adding sterile distilled water (Acott and Labuza, 1975). The amount of water added was calculated theoretically on the basis of the original moisture content of the seeds. Moisture content was determined by

drying about 10 g of each sample of seeds in an electrical oven at 100-105°C untile a constant weight (Mallick and Nandi, 1979). Samples were kept at 4°C for 5 days, and after equilibration, a volume of *A. flavus* spore suspension (10^8 spores/ml) was added to the precondition- ed seeds so that these seeds contained 10^6 spore / ml. The inoculated samples were maintained in separate well closed flasks at 28°C for 30 days. At the end of incubation period, aflatoxin B_1 production was determined and the samples were analyzed for the protein, lipids and carbohydrates.

Radiation effects on aflatoxin B_1 production by *A.flavus* in sterilized and non-sterilized seeds:

Samples (300g) of wheat, soyabean and fababean seeds free from aflatoxins with moisture content of 25% in conical flasks were irradiated in air with Russian ^{60}Co gamma cell, NCRRT, Cairo, Egypt (dose rate 200 Gy/min) using 10 kGy as sterilized dose for seeds (El-Zawahry *et al.*, 1991). The seeds were inoculated with 10^8 spores of *A. flavus* per gram seeds then the samples were irradiated at dose levels of 0, 1, 3 and 5 kGy, and were maintained at 25°C for 30 days. At the end of incubation period, aflatoxin B_1 levels and number of *A. flavus* colony forming units (cfu) were determined and the samples were analyzed for the total protein, lipids and carbohydrates.

Aflatoxin measurement:

Aflatoxin B_1 extraction and detection on wheat, soyabean and fababean seeds were carried out using the method of AOAC (1990). Thin layer chromatography (TLC) was performed with precoated glass plates of silica gel G (Merck) using acetone: chloroform (1:9 v/v), air dried plates were examined under UV light (366 nm). Aflatoxin B_1 was characterized by comparing the R_f value and fluorescence of external standard aflatoxin B_1 with the unknown samples. Recovery studies were performed by adding 30 µg/kg of aflatoxin B_1 to wheat, soyabean and fababean samples. Average recovery of aflatoxin B_1 was found to be 92, 85 and 76%, respectively. The quantity of aflatoxin B_1 was determined using UV spectrophotometer according to (AOAC 1990). Confirmation of aflatoxin positive samples were made by trifluoroacetic acid directly to TLC plates (Anon., 1975).

Chemical evaluations:

Sample of seeds were analyzed for dry matter, protein (P), lipids (L), crude fiber (CF) , total carbohydrates (C) and ash using the American Association of Cereal Chemists (AACC, 1983) rapid method on dry matter basis.

Statistical Analysis:

Least significant difference (LSD) was used to compare treatment means (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

The genera and distribution of the fungi isolated from initial samples of wheat, soyabean and fababean are shown in Table (1).

Table1: Percent occurrence of fungal genera and species in surface disinfected and non-disinfected wheat, soyabean and fababean seeds (5-samples).

Fungal species	Wheat seeds		Soyabean seeds		Fababean seeds	
	Non-disinfected %	Surface-disinfected %	Non-disinfected %	Surface-disinfected %	Non-disinfected %	Surface-disinfected %
Alternaria sp.	25	0	18	0	19	0
Aspergillus flavus	75	100	62	92	55	75
A. niger	60	80	60	90	50	70
A. candidus	12	0	0	0	8	0
A. nidulans	0	0	26	0	0	0
A. ochraceus	44	0	36	0	12	0
A. fumigatus	34	0	30	0	0	0
Cladosporium sp.	48	0	56	0	30	0
Epicoccum sp.	8	0	6	0	20	0
Fusarium sp.	60	0	43	0	20	0
Penicillium sp.	18	0	22	0	40	0
Mucor sp.	30	0	40	0	0	0
Helminthosporum sp.	0	0	15	0	0	0
Rhizopus sp.	18	0	16	0	12	0

Alternaria. Aspergillus, Cladosporium, Epicocum, Fusarium, Penicillium, Mucor, Helminthosporium and *Rhizopus* were predominant fungal genera isolated from all non disinfected samples. *Aspergillus flavus* and *Aspergillus niger* were found in 100 to 75% of surface disinfected wheat, soyabean and fababean samples. Species of *Aspergilli, Penicilli, Fusarium, Alternaria, Cladosporium, Helminthosporium, Curvularia, Epicoccum* and *Verticillium* were common and widespread in soil, litter as well as many food and feed ingredients (Smyk *et al.*, 1989; Aziz *et al.*, 1990 and EL-Zawahry *et al.*, 1991). *Aspergillus flavus* and *A. parasiticus* were observed as the main popular fungal species recovered from food and feed commodities (EL-Khadem *et al.*, 1983, EL-Far *et al.*, 1992, Hassan and Aziz, 1998 and Mahrous *et al.*, 2001).

The results recorded in Table (2) point that 28 (57.1%) out of 49 *A. flavus* isolates from wheat, soyabean and fababean produced aflatoxin B_1. Meanwhile eighteen isolates (64.3%) *A. flavus* from wheat samples produced aflatoxin B_1 at an average from 300 to 2600 µg kg^{-1}. Whereas 7 isolates (53.8%) from soyabean samples produced aflatoxin B_1 at an average from 85 to 1400 µg kg^{-1} and only 3 isolates (37.5%) from fababean produced aflatoxin B_1 at levels from 70 to 100 µg kg^{-1}. Nowadays aflatoxin formation is a phenomenon of most isolates of *A. flavus* group (Rustom, 1997) and aflatoxins were found to contaminate a wide variety of important agricultural products as wheat, barley, peanut, maize, rice, cotton seed and seasam (Aziz *et al.*, 1994, Farag *et al.*, 1995 and Shahin and Aziz 1997 and Mahrous *et al.*, 2001).

Table 2: Production of aflatoxin B_1 by *Aspergillus flavus* isolated from wheat, soyabean and fababean seeds.

Seed commoditiy	No. of tested isolates	No. of +ve isolates	Average of aflatoxin B_1 µg/kg
Wheat	28	18 (64.3%)	300-2600
Soyabean	13	7 (53.8%)	85-1400
Fababean	8	3 (37.5%)	70-100
Total	49	28 (57.1%)	

Table (3) shows the chemical composition of healthy and *A. flavus* infected wheat, soyabean and fababean seeds incubated for 30 days. The data show that the growth of *A. flavus* behaved differently according to the principal chemical constituents of the seeds. For instance, *A. flavus* caused an increase in protein content of wheat, soyabean and fababean and there was a significant decrease in the carbohydrate and lipid contents of all seeds after 30 days of incubation at 28°C. These results demonstrate that the fungus utilized the basic compound of seeds for its growth. The differences in crop composition were mainly due to the influence of the pathway to use major energy source of each seed whether carbohydrate (soyabean) or lipid (wheat).

Table 3: The chemical composition (% on DM basis) of healthy and *A. flavus* infected wheat, soyabean and fababean after 30 days of incubation at 28°C.

Seeds Composition	Wheat*		Soyabean*		Fababean*	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
Moisture (%)	6.8 ± 0.06	8.81 ± 1.6	7.30 ± 0.06	8.41 ± 0.13	6.71 ± 0.11	9.70 ± 1.6
Protein (Nx6.25) (P)	16.36± 3.10	22.3±1.97	38.26±1.80	48.20±3.3	28.41±2.60	39.81±4.50
Lipids (L)	2.91±0.50	1.60±0.35	28.70±2.5	26.80±1.65	1.95±0.18	1.60±0.11
Carbohydrates (C)	75.61±4.80	64.11±5.80	23.65±6.70	16.71±1.51	59.68±4.1	49.40±1.45
Crude fiber	3.79±1.12	3.54±1.8	5.13±1.41	5.90±1.60	5.77±1.33	5.60±1.61
Ash	1.33±0.02	3.45±0.02	4.29±0.03	2.39±0.02	4.19±0.03	3.19±0.03

* Values are means of 4 replicates ± SD.

Table-5: Influence of gamma radiation on *A. flavus* population and aflatoxin B_1 production in wheat, soyabean and fababean seeds stored for 30 days at 25°C and R.H. 95% (moisture 25%).

Radiation dose kGy	Wheat[a]				Soyabean[a]				Fababbean[a]			
	Intial		30 days**		Intial		30 days**		Intial		30 days**	
	Log_{10} cfu/g	Aflatoxin B_1 conc. μgkg^{-1}	Log_{10} cfu/g	Aflatoxin B_1 conc. μgkg^{-1}	Log_{10} cfu/g	Aflatoxin B_1 conc. μgkg^{-1}	Log_{10} cfu/g	Aflatoxin B_1 conc. μgkg^{-1}	Log_{10} cfu/g	Aflatoxin B_1 conc. μgkg^{-1}	Log_{10} cfu/g	Alatoxin B_1 conc. μgkg^{-1}
0	6.15	0	7.65	280±3.5	5.90	0.0	6.94	210±4.2	5.85	0.0	6.83	100±2.2
1	4.08	0	6.90	220±1.8	3.50	0.0	3.65	125±1.6	2.60	0.0	3.79	96±1.3
3	1.80	0	2.08	85±1.5	1.60	0.0	1.30	45±3.5	1.30	0.0	1.61	96±1.8
5	0.00	0	0.00	0.0	0.00	0.0	0.0	0.0	0.00	0.0	0.0	30±1.8

[a] : Values are means of three replicates of two repeated experiments

Values decreased significantly ($P<0.05$) with increasing doses of radiation.

*: cfu/g = colonies forming units/g.

**: Values increased significantly ($P<0.01$) with increasing storage periods.

In a previous study, Farag (1990) reported that *Aspergillus flavus* infection caused no change in lipid content but an increase and decrease in protein and carbohydrate contents, respectively of wheat kernls (the starchy crop) were happened and the fungus caused no change in the protein content of sesame seeds (high lipid content), but it increased and decreased the carbohydrate and lipid contents, respectively. In general, it was clear that the activities of amylase, lipase and protease were much higher in infected seeds than in the healthy one (Russell *et al.,* 1982 and Park and Bullerman, 1983), these observations explain our present data (Table-3) which showed that *A. flavus* utilized the basic compound of seeds for its growth during the incubation period.

Table (4) shows aflatoxin B_1 production by *A. flavus* in wheat, soyabean and fababean seeds. Wheat seeds infected with *A. flavus* generally contained greater amounts of aflatoxin B_1 (218 $\mu g kg^{-1}$) than soyabean (200 $\mu g\ kg^{-1}$) and fababean (85 $\mu g\ kg^{-1}$) after 30 days of incubation at 28°C. Sterilization process plays a dual function by causing the breakdown of the outer shell and destroying some of the competitive microorganisms associated with seed crops (Hassan and Aziz, 1998). Cuero *et al.,* (1986 and 1988) stated that aflatoxin production from *A. flavus* is dependent on the competing organisms and water activity. The higher levels of aflatoxin that resulted from incubation on seeds could be explained as sterilization reducing competing microbiota resulting in better growth and toxin production by *A. flavus,* as shown in Table (4).

Table-4: Aflatoxin B_1 ($\mu g\ kg^{-1}$) production by *A. flavus* in artificially inoculated wheat, soyabean and fababean seeds after 30 days of incubation at 28°C.

Commodity	Incubation periods (days)*			
	0	10	20	30
Wheat	0	35±.6.1	125±2.1	218±2.0
Soyabean	0	28±2.8	110±2.6	200±3.8
Fababean	0	18±1.4	38±3.5	85±4.1

*** Values are means of three replicates of two repeated experiments ±SD.**
Aflatoxin concentrations increased significantly ($P < 0.05$) with storage .

Aflatoxin contamination of grains is difficult to predict because, it depends on a complex interaction factors such as temperature, moisture, the kind of seeds, endogenous fungal species, storage time, storage history, type of transit and transit time (Hill and Lacey, 1984 ; Magan and Lacey, 1984, Hassan and Aziz, 1998 and Mahrous *et al.,* 2001). The high incidence of aflatoxin B_1 in wheat grains in the present investigation was in accord with several investigators (Cuero *et al.,* 1988, Abramson *et al,* 1990, Mills, 1990, Sinha and Sinha, 1991 and Mahrous *et al.* 2001) who demonstrated that *A. flavus* could produce maximum concentration of aflatoxin B_1 (686-4318 $\mu g\ kg^{-1}$) in wheat grains with 20% moisture after 20 days at 30°C and RH of 92.04% as compared with other seeds..

Table (5) shows that when *A. flavus* (10^6 spores/gram) was inoculated artificially into sterile wheat, soyabean and fababean then irradiated at different dose levels, 1, 3 and 5 kGy it was noticed that the irradiation dose level 5kGy completely inhibited the fungus immediately after irradiation and during the 30 days of incubation at 28°C.

Also, Table (5) clears that by increasing the irradiation dose level up to 3 kGy there was a significant decrease in the concentrations of aflatoxin B_1 and no detection of the toxin in all irradiated seeds at a dose level of 5 kGy as compared to the control seeds (100-280 µg Kg^{-1}) after 30 days of incubation at 28°C. Gamma irradiation is known to cause injury to microorganisms and has been widely reported to prevent or delay food spoilage (Aziz *et al.*, 1990; Gharib and Aziz, 1995; Hammad *et al.*, 1996 and Youssef *et al.*, 1999). There has been increasing interest in the use of ionizing radiation for reducing the occurrence of mycotoxin in different food and feed products (Youseef *et al.*, 1995; Aziz *et al*, 1997 and Hassan and Aziz, 1998). Recently, Shahin and Aziz (1997) found that, by increasing the radiation doses, the viable population and aflatoxin B_1 production by *A. flavus* NRRL 5520 in peanut were decreased, and no growth and no detection of aflatoxins were occurred in peanut after treatment with 4.0 kGy.

Aspergilli are known to be present in pre-harvest and post-harvest grains and their presence affects growth and mycotoxin production in storage (Abramson *et al.*, 1990 and Mahrous *et al.*, 2001). Hassan and Aziz (1998) found that a dose of 4.0 kGy eliminated all viable fungi in maize samples, aflatoxin B_1 production was decreased with increasing levels of irradiation and was negligible at 4.0 kGy. Also, the authors revealed that when maize was inoculated after irradiation and stored, the spore counts and aflatoxin levels were higher than control in unirradiated and inoculated controls after 30 days and the natural competitive microflora prevented growth and thus limited higher concentrations of aflatoxin in maize.

Table (6) shows that after 30 days of incubation at 28°C the growth of *A. flavus* in the unirradiated seeds caused an increase in the protein content to 26.50, 48.31 and 49.46% for the unirradiated wheat, soyabean and fababean seeds, respectively. By increasing the irradiation dose levels there was a positive relation between the decrease in the *A. flavus* colony-forming units/g and the decrease in the protein content for all seeds. For instance, due to the decrease of *A. flavus* growth there was a significant increase in the carbohydrate contents of the irradiated seeds. The present results demonstrate that *A. flavus* growth utilized the basic compounds of the seeds especially carbohydrates for its growth and aflatoxin B_1 production and that is why at the end of the incubation period there was a significant increase in the protein content which includes both the protein of the substrate and the microbial protein and hence a decrease in the carbohydrates content of the seeds. In general, most fungi which infect seeds during storage led to a change in the chemical composition as to an increase and / or decrease in the protein, carbohydrate and lipids (Cuero *et al.*, 1988, Farag, 1990 and Youssef *et al*, 1995)

Table-6: Effect of *Aspergillus flavus* growth on the chemical composition (% on DM basis) of the gamma-irradiated seeds after 30 days of incubation at 28°C.

Agricultural commodities	Irradiation Doses kGy	Dry Matter %	Protein * %	Lipids * %	Carbo-hydrates * %	Ash %
Wheat	0	89.7 ± 0.26[a]	26.50±2.50 [a]	2.85±0.40 [a]	66.11±3.40 [a]	3.33 ± 0.20 [a]
	1	91.8 ± 0.40[a]	26.81±1.86 [a]	2.41±0.66 [b]	65.80±2.80 [a]	3.11 ± 0.18 [a]
	3	91.7 ± 0.30[a]	18.31±1.66 [b]	2.55±0.41 [b]	76.12±2.70 [b]	3.12 ± 0.14 [a]
	5	92.3 ± 0.41[a]	17.91±1.45[b]	2.66±0.41 [b]	76.61±2.70 [b]	3.14 ± 0.30 [a]
Soyabean	0	92.60 ± 0.39 [a]	48.31±2.50 [a]	26.66±1.80 [a]	19.80±2.50 [a]	4.81 ± 0.70 [a]
	1	92.70 ± 0.41 [a]	46.71±2.71 [a]	26.89±1.40 [a]	19.80±2.11 [a]	3.99 ± 0.33 [a]
	3	91.50 ± 0.60 [a]	46.41±1.80 [a]	26.77±1.77 [a]	20.56±1.76 [a]	4.20 ± 0.41 [a]
	5	91.60 ± 0.40 [a]	38.60±1.80 [b]	27.21±2.60 [a]	21.30±1.89 [b]	4.11 ± 0.11 [a]
Fababean	0	90.30± 0.11 [a]	49.46±2.80 [a]	1.88±0.06 [a]	46.80±2.80 [a]	3.17 ± 0.21 [a]
	1	91.40 ± 0.12 [a]	48.80±1.88 [a]	1.88±0.06 [a]	47.11±2.22 [a]	3.41 ± 0.11 [a]
	3	90.70 ± 0.15 [a]	27.50±1.61 [b]	1.66±0.03 [b]	66.15±3.50 [b]	3.16 ± 0.40 [a]
	5	91.20 ± 0.16 [a]	17.24±1.80 [b]	1.60±0.02 [c]	66.11±3.90 [b]	3.14 ± 0.11 [a]

* :Values are means of 4 observations ± SD Means with the same letter are not significantly different (P > 0.05)

Initial chemical composition of wheat seeds: 16.36% protein, 2.91% lipids and 75.61% carbohydrates.

Initial chemical composition of soyabean seeds: 38.26%, protein, 28.7% lipids, and 23.65% carbohydrates.

Initial chemical composition of fababean seeds: 28.4% protein, 1.95% lipids and 59.68% carbohydrates.

The results of the present study revealed that irradiation at dose levels of 1 or 3 kGy did not reflect appreciable changes in the chemical composition of wheat, soyabean and fababean seeds when compared with that of raw seeds. The present data are in agreement with those recorded by several investigators (Hammad, 1985; Farag and Diaa El-Din, 1998 and Seda *et al,* 2002).

It was concluded before that gamma irradiation has been proved as an effective and safe treatment and could be used as a method to control mould grown in grain and grain products. It is assumed that no toxicological conservation of any irradiated food commodity. Hence, food treated in this way no longer needs to be tested for toxicity (Youssef *et al.,* 1995).

In the present study, it can be suggested that caution should be taken into considerations when ever grains are to be stored prior to use, because of the natural spontaneous occurrence of aflatoxigenic strains of *Aspergillus* as one of the normal seeds microflora. Observations in this study reported that mould growth behaved differently according to the principal chemical constituents of the seeds, wheat (carbohydrate seeds), soyabean (oily and proteineous seeds) and fababean (proteineous seeds). In addition the chemical composition of seeds plays a great role in the induction of mycotoxin by toxigenic moulds and gamma irradiation can control fungal infection and aflatoxin B_1 production in seeds. Further studies are requied to declare the chemical changes in gamma-irradiated fungal cells and food materials and the accumulation pathway of mycotoxins in seeds under different environmental conditions.

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تأثير أشعة جاما والمكونات الكيميائية لبعض بذور المحاصيل على إنتاج الأفلاتوكسين بواسطة فطر الأسبرجلس فلافس

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فى هذا البحث تم دراسة تأثير أشعة جاما والتركيب الكيميائى لبعض البذور على إنتاج الأفلاتوكسين ب، بواسطة فطر الأسبرجلس فلافس. ولقد لوحظ فى هذه الدراسة اختلاف سلوك نمو الفطر الملوث للبذور باختلاف المكونات الأساسية لهذه البذور. ولوحظ أن إصابة البذور بفطر الأسبرجلس فلافس أدت إلى زيادة نسبة البروتين وخفض نسبة الكربوهيدرات والدهون فى جميع البذور.

ولقد كانت الجرعة الإشعاعية ٥ كيلو جراى كافية لتثبيط نمو فطر الأسبرجلس فلافس وإنتاج الأفلاتوكسين تماما. ولقد أظهرت النتائج أنه لم يكن هناك أى تغير فى المركبات الكيميائية الأساسية للبذور كالبروتين والدهون والكربوهيدرات نتيجة لاستخدام الإشعاع بجرعة حتى ٣ كيلوجرام.

وفى هذا البحث لوحظ استخدام فطر الأسبرجلس فلافس للمكونات الأساسية للبذور وخاصة الكربوهيدرات فى نموه وانتاجه للأفلاتوكسين حيث تم ترتيب البذور حسب إنتاج الأفلاتوكسين ترتيبا تنازليا القمح يليه فول الصويا ثم الفول البلدى.