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Research Article

STUDY OF THE STRUCTURAL COMPARISON OF ASPERGILLUS *FLAVUS* INOCULATED IN PEANUT GRAINS AND INDUCED BY CS-137 GAMMA RADIATION AND ELECTRON BEAM

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ABSTRACT

This study compared the phenotypes of Aspergillus *flavus* CMT 00079 inoculated in peanut grains after induction of Cs 137 gamma radiation and electron beams. Peanut grains were distributed in Petri dishes and inoculated with the fungal strain and incubated in a BOD germination chamber at 25ºC for 5 days. After 24 hours, the plates were irradiated with Cs137 and absorbed doses of 1.0 KGy with 0.5kGy increase to 8.0 KGy. In electron beam irradiation the absorbed doses were 6.0; 7.0; 7.5 and 8.0 KGy. In both sources the standard 0 KGy plate was maintained. The inactivation doses of Cs 137 strains ranged from 4.5 to 8.0 KGy and with electron beam from 6.0 to 8.0 KGy. Between days 1; 7; 15 after irradiation. Within 15 days after irradiation the subcultures in isolated and irradiated nutrient medium recovered their fungal growth from 1 to 7.5 KGy with CS 137 and 6.0 to 7.5 KGy with electron beam. The results showed that the absorbed dose of 8.0 KGy was sufficient to inactivate or eliminate Aspergillus *flavus*.

INTRODUCTION

Aspergillus is a genus composed of more than 200 accepted anamorphic species (PITT, 2000), with teleformism described in nine different genera (SAMSON & PITT, 2009). This genre is divided into 6 subgenres which are further divided into sections (Klich, 2002).

Although the genus contains more than 280 species already studied for several centuries, its system is still in a state of flux, always evolving (SAMSON & PITT, 2009). The gender It is easily identified by the presence of the gallbladder at the end of the conidiophore and the presence of a podal cell, but species identification and differentiation is complex and traditionally based on morphological characteristics. Among the macromorphological characteristics considered are conidial and mycelial staining, colony diameter, colony reverse staining, exudate production and soluble pigments, presence of sclerotia.

Changes in ecological factors that impact the peanut's microbiota occur after the harvesting, drying, and storing processes when the *Aspergillus* spp. and *Penicillium* spp. fungi become predominant (SANTOS *et al*., 2013).
The genus Aspergillus is considered the major cause of deterioration of agricultural products, both before and after harvest. In addition to food spoilage, of concern is the variety of mycotoxins that can be produced. It is the most common toxigen affecting the food chain and may contaminate products such as peanuts, walnuts, sorghum, soybeans and other oilseeds (KLICH, 2002). *A. flavus* is of particular importance due to its impact on agriculture and health. human (DURAN *et al*., 2007).

A. flavus is a worldwide occurrence, apparently being more common in cultivated than uncultivated soils. This species is capable of colonizing decaying vegetables, grains, seeds and many other substrates, including food and animal feed (GONÇALVES, *et al*. 2013; ROSA, 2002).

Grown in over 80 countries in both hemispheres, especially in tropical regions at 30ºN and 10ºS latitudes, peanut grain is a highly energetic food (582 calories .100g-[1]). Its seeds are rich in oil (48.7%), consisting of 80% unsaturated fatty acids, among them oleic and linoleic (COELHO, 2003). Oil and its proteins have high nutritional quality, which determines the significant economic value in first world countries and in those that have limitations of protein supplementation in the diet (MACEDO, 2004).
Peanuts also contain important amounts of vitamin E, vitamin B1 and folic acid (MACEDO, 2004). Regarding minerals, it presents high concentrations of potassium, phosphorus and

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zinc (FREIRE, 2005). It also has great importance in human food, which is related to the fact that the grains have a pleasant taste, good digestibility and little difference between raw food, cooked or submitted to any other treatment.

To reduce or eliminate microbiota on different substrates such as peanuts, rice, oats, wheat and corn, gamma irradiation has been used (BORGES, 2011; FARKAS, 2011; BENTO *et al*., 2012; COSTA, 2013). Gamma radiation has a high power of penetration of electromagnetic waves that pass through food without leaving residues and have advantages compared to other disinfection treatments, such as those using chemicals. The electron beams are generated by accelerators. Electrons are electrically accelerated into a vacuum chamber at a very high speed, close to that of light.

The food irradiation method contributes to the marketing of safe products, nationally and internationally, with direct benefits throughout the supply chain (International Consultative Group on Food Radiation, 1995).

The present study evaluated the effects of gamma and electron beam radiation on *Aspergillus flavus* strains inoculated in peanut grains. The macromorphology of the fungus was observed in order to evaluate the parameters that could cooperate for its inactivation or elimination.

MATERIAL AND METHODS

Contamination of the Samples

Shelled peanut grains were purchased in supermarkets. The peanut samples were hydrated in the rheology and milling laboratory, the current EMBRAPA Cereal Laboratory. After 3 days the water activities were verified to confirm the ideal pattern for fungus inoculation.

Aspergillus *flavus* CMT 00079 was reactivated in PDA (Patato Dextrose Agar) medium and distributed into Petri dishes in triplicates. Incubated for 10 days in a BOD (Biological Oxygen Demand) germination chamber, Fanem model 347, at 25°C. Mycological analyzes were performed at the Taxonomy, Biochemistry and Fungus Bioprospecting Laboratory (LTBBF) at IOC / FIOCRUZ.

Irradiation of the Samples

Peanut kernels were inoculated with grown cultures of *Aspergillus flavus* CMT 00079 and incubated in a BOD germination chamber at 25°C for 5 days.

On the sixth day, peanuts were irradiated with gamma radiation at the doses of 0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 kGy in the Arm Technological Center (CTEx). The dose rate provide by irradiator was 26,66 Gy/min, at 33°C.

On the sixth day, the peanuts were irradiated with electron beam in the doses 0, 6.0, 7.0, 7.5 and 8.0 kGy in the Aceletron Industrial Irradiation. The electron beam was generated in a cathodic semiconductor, using electric energy and accelerated by the LINAC (electron beam irradiator). The dose rate of the linear accelerator remained constant. The dose applied to the product could be varied by adjusting the speed of the conveyor belt, thereby controlling the time of exposure of the product to the bundle. Two linear electron accelerators (LINAC) of 18 kW of power and 10MeV of energy constituted the radiating installation.

Cultivation of Aspergillus flavus

Grown cultures of Aspergillus *flavus* CMT 00079 were inoculated into previously sterilized peanut kernels, distributed in Petri dishes and incubated in BOD germination chambers at 25 ° C for 5 days. On the sixth day the peanuts were irradiated. The CS 137 gamma irradiator provided the dose rate of 26.66 Gy / min. at 33 ° C. The absorbed doses were 0; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5 and 8.0 KGy.

In ACELETRON industrial irradiation, two 18 kW linear and 10 MeV linear electron accelerators (LINAC) were the radiant installation. The electron beam was generated in a cathodic semiconductor using electrical energy and accelerated by the LINAC (electron beam radiator). The linear accelerator dose rate remained constant. The dose applied to the product can be varied by adjusting the speed of the conveyor belt, thereby controlling the exposure time of the product to the beam. The absorbed doses were 0; 6.0; 7.0; 7.5 and 8.0 Kgy.

Isolation of Aspergillus flavus fungal colonies

After gamma and electron beam irradiations, the plates were taken to the Taxiomyology, Biochemistry and Fungus Bioprospecting Laboratory (LTBBF) - IOC / FIOCRUZ, where the irradiated grains were directly plated.

To verify the presence or absence of growth of fungal colonies and other characteristics, after irradiation, irradiated peanut grains were removed from each plate and inoculated directly into Petri dishes containing BDA culture medium. The plates were incubated in a BOD germination chamber at 25 ° C for up to 15 days. In parallel the inoculation of the Petri dishes was inoculated in test tubes with BDA (Potato Destrose Agar) culture medium. The growth of colonies that were monitored on days 1, 7 and 15 after irradiation was followed.

Observation of Aspergillus flavus fungal colonies

Colonies growing in the test tubes were inoculated at 3 equidistant points from the Petri dishes containing the MEA (Agar Malt Extract) culture medium and incubated for 7 days to observe the macroscopic characteristics of the isolated colonies.

On the 7th day, colony growth readings were taken with the aid of an electronic caliper and observation of the color, general appearance of the colony and its reverse to evaluate fungal radiosensitivity. The morphological characteristics of the colonies were attributed based on the descriptions recommended by Klich (2002).

RESULTS AND DISCUSSION

Gamma Irradiation with Cs 137

It was observed that the tubes on the 1st and 7th day after irradiation grew at: 0; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0 KGy, with the probability of inactivation occurring at doses from 4.5 to 8.0. In the tubes of the 15th day after irradiation there was growth in 0; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0; 5.5; 6.0; 7.0 and 7.5 KGy, suggesting inactivation at the 8.0 KGy dose.

Electron beam irradiation

Colonies grew in the tubes on the 1st and 7th days after irradiation only at the control dose 0 KGy, with the probability of inactivation at the doses of 6.0; 7.0; 7.5 and 8.0 KGy. At the15th day after irradiation there was growth at 0; 6.0; 7.0 and 7.5 KGy, suggesting inactivation at the dose of 8.0 KGy. It has

been observed that colonies shrink and take time to grow back, but at 8.0 KGy they no longer grow.

Table 1 Growth *A. flavus* CMT 00079 irradiated with Cs 137

kGy Dose	Day 1	Day 7	Day 15
0.0;	+	+	+
1.0	+	+	+
1.5	+	+	+
2.0	+	+	+
2.5	+	+	+
3.0	+	+	+
3.5	+	+	+
4.0	+	+	+
4.5	-	-	+
5.0	-	-	+
5.5	-	-	+
6.0	-	-	+
6.5	-	-	+
7.0	-	-	+
7.5	-	-	+
8.0	-	-	_

+ Growth; **- No growth**

Table 2 Growth *A. flavus* CMT 00079 electron beam irradiated

kGy Dose	Day 1	Day 7	Day 15
0.0	+	+	+
6.0	-	-	+
7.0	-	-	+
7.5	-	-	+
8.0	-	-	-

+ Growth; **- No growth**

Table 3 Diameter of *A. flavus* colonies (CMT 00079) incubated for 7 days on Cs137

kGy Dose	Diameter mm
0.0	65.02
1.0	62.72
1.5	57.89
2.0	56.14
2.5	55.50
3.0	53.17
3.5	50.66
4.0	50.23
4.5	46.32
5.0	36.72
6.0	19.17
7.0	12.53
8.0	N.G.

The measurements were taken in triplicate and their respective averages calculated.

NC = Not grown

It was observed that from 0 to 4.0 KGy A. *flavus* colonies behaved within the normal range, tending to decrease with increasing dose. Such shrinkage effect was accentuated, being that the colonies presented small dimensions of 4.5 to 7.5 KGy, the diameters of the colonies differed, because they were below the minimum limit established in studies published in the literature. At 8.0 KGy no growth was observed.

Table 4 Diameter of *A. flavus* colonies (CMT 00079) incubated for 7 days on electron beam irradiated MEA.

kGy Dose	Diameter mm
0.0	65.02
6.0	16.62
7.0	8.25
7.5	5.00
8.0	N.G.

The described measurements were made in triplicates and their respective averages were calculated.

NC = Not grown

According to the measurements performed (Table 4), a reduction in the diameter of *A. flavus* colonies (CMT 00079) was observed with increasing dose. At 6.0 kGy the retraction is already significant, further reducing by 7.5 kGy. From 6.0 to 7.5 kGy, the diameter of the colonies were smaller than those described by Klich (2002). From 8.0 kGy there was no growth.

Table 5 presents the measurements of diameters with statistical treatment for *A. flavus* using gamma rays and electron beams.

Table 5 Dose X Diameter - *A. flavus* CMT 00079 (gamma ray and electron beam)

kGy Dose	Standard Diameter (65.02 mm=1) Gamma Cs 137	Electron Beam Stander Diameter	Average Diameter
0.0	1	1	1
1.0	0.9646	N.O	0.9646
1.5	0.8903	N.O	0.8903
2.0	0.8634	N.O	0.8634
2.5	0.8536	N.O	0.8536
3.0	0.8177	N.O	0.8177
3.5	0.7791	N.O	0.7791
4.0	0.7725	N.O	0.7725
4.5	0.7124	N.O	0.7124
5.0	0.5647	N.O	0.5647
5.5	0.3880	N.O	0.3880
6.0	0.2948	0.2556	0.2752
6.5	0.2381	N.O	0.2381
7.0	0.1927	0.1269	0.1598
7.5	0.1300	0.0769	0.1035

NO = Not observed

In the case of the normalization curve, the diameter of 65.02 mm was considered 1.0000.

In this work we observed morphological variations between control and irradiated strains, such as color, texture and reverse colony (Table 6).

It was found that the macroscopic changes found at doses from 5.0 to 7.5 KGy are not within the observed variations for the species according to the literature.

Table 6 Macromorphological characteristics of *A. flavus* CMT 00079 colonies irradiated with Cs 137

Dose kGy	Physical characteristics and coloration of *A. flavus* (CMT 00079) colonies isolated in MEA culture medium
0,0-2,0	Cologne with flocculate texture, high center, olive green; white mycelium with reverse of yellowish olive green to opaque yellow. Presence of sclerotia in brown color.
2,5	Colony with flocculate texture raised center, olive green; white mycelium with reverse of yellowish white to brownish yellow. Presence of sclerotia in brown color.
3,0	Cologne with flocculate texture, high center, olive green; white mycelium with reverse of olive green brownish to yellowish white. Presence of sclerotia in brown color.
3,5 – 4.0	Cologne with flocculate texture, high center, olive green; white mycelium with reverse from olive green to opaque yellow. Presence of sclerotia in brown color.
4,5	Cologne with flocculate texture, high center, olive green; white mycelium with reverse from brownish green to opaque yellow. Presence of sclerotia in brow color.
5,0	Cologne with flocculate texture, olive green brown; white mycelium with reverse from brownish-green to a whitish yellow back. Discrete presence of brown sclerotia.
5,5	Colony with flocculate texture, gray yellow, yellowish white mycelium with reverse brownish orange to whitish yellow.
6.0	Cologne with flocculate texture, olive green; yellowish white mycelium with reverse from gray green to pastel green.
6.5	Cologne with flocculate texture, olive green; yellowish white mycelium with reverse from gray olive green to gray yellow.

7.0	Cologne with flocculate texture, opaque green; opaque green mycelium with reverse yellowish gray green to yellow opaque.
7.5	Cologne with velvety texture, light green; opaque green mycelium with reverse gray green.

In plates with absorbed doses of 0 and 2.5 kGy, the presence of sclerotia was clearly visible in the colonies. From 3.0 to 4.0 kGy there was a slight decrease in the presence of light brown sclerotia. In the 4.5 and 5.0 kGy plates, a large decrease in light brown sclerotia was observed. From 5.0 kGy on, sclerotia was barely noticeable. These macroscopic changes are within the observed variations for the species, according to the literature.

Table 7 Macro morphological characteristics of *A. flavus* (CMT 00079) colonies irradiated with electron beam

Dose kGy	Physical characteristics and coloration of *A. flavus* colonies (CMT 00079) isolated in MEA culture medium
0,0	Cologne with flocculate texture, high center, olive green, white mycelium with reverse yellowish olive green to opaque yellow. Presence of sclerotia in brown color.
6,0	Cologne with flocculate texture; pastel green, white mycelium with reverse of gray green to pastel green. Presence of sclerotia in brow color.
7,0	Cologne with flocculate texture; olive green, White mycelium with reverse of gray green. Presence of sclerotia in brow color.
7,5	Cologne with velvety texture; light green, discrete white mycelium and pale green reverse. Discrete presence of sclerotia in brow color.

In plates with absorbed doses of 0; 6.0 and 7.0 kGy were observed in the colonies the presence of light brown sclerotia. At 7.5 kGy the sclerotia was not noticeable. These macroscopic changes are within the range normally observed for species, according to the existing literature.

CONCLUSION

This study broadened the macromorphology overview of the Aspergillus *flavus* (CMT 00079) species irradiated with different doses of gamma rays (Cs 137) and electron beam. The probability of survival of irradiated A. *flavus* in peanut substrates decreases with the absorbed dose of ionizing radiation. Radiation caused a strong stress to the colonies, which immediately retracted, such retractions being larger at higher doses. After a period of recovery, they may or may not expand again, depending on their radioresistance and vitality, and whether or not inactivation occurs. Gamma irradiation, compared to the electron beam, altered the morphology of the toxigenic fungus Aspergillus flavus, causing a retraction of the mycelium and other structures, increasing with the absorbed radiation dose. The required dose of gamma radiation (Cs137) or electron beam to inactivate the fungal contamination of A. *flavus* peanuts was 8.0 kGy.

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