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USE OF ELECTRON BEAM ON AFLATOXINS DEGRADATION IN COCONUT AGAR

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ABSTRACT

The fungi *Aspergillus flavus* are capable of producing toxic metabolites, such as aflatoxin, that is one of the most important human carcinogens, according to the "International Agency for Research on Cancer". The aim of this study was to compare the effect of electron beam irradiation on degradation of aflatoxin B_1 present in laboratorial residues with a dose of 0 kGy and 5.0 kGy. The fungi were cultivated in potato dextrose agar (PDA) for 7 days and transferred to a coconut agar medium, incubated at a temperature of 25 ºC for 14 days to produce the laboratorial wastes (coconut agar) containing aflatoxins. The samples were conditioned in petri dish for radiation treatment of contaminated material and processed in the Electron Accelerator with 0 kGy and 5.0 kGy. Aflatoxin B_1 was extracted with chloroform and separated on a thin layer chromatography plate (TLC) with chloroform: acetone (9:1). All the control and irradiated samples were analyzed in a Shimadzu Densitometer. The detection limit of this methodology is 0.1 μg kg^{-1}. The results indicate that the irradiated samples had a reduction of 75.49 % in the analyzed dose.

1. INTRODUCTION

The moulds *Aspergillus flavus* and *Aspergillus parasiticus* are important pathogens of crops, because they produce aflatoxins [1]. There are four naturally aflatoxins, B_1, B_2, G_1 and G_2 and the aflatoxin B_1 is considered one of the most important hepatocarcinogens by "International Agency for Research on Cancer", being classified on group 1. Aflatoxin is a secondary fungal metabolism, and is considered one of the most important human carcinogens [2,3,4]. Aflatoxins are bifuranocumarin mycotoxins and appear fluorescent under ultraviolet light [5,6]. The letter "B" and "G" refer to the blue and green fluorescent produced by aflatoxins B_1, B_2, G_1 and G_2 under UV light, and the numbers "1" and "2" show the major and minor compounds [7]. Aflatoxins are the most important mycotoxins and are produced by four species of fungi of the genus *Aspergillus*. These species are *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius* and *Aspergillus pseudotamarii*, but only *A. flavus* and *A. parasiticus* has economic importance because of the large capacity to develop in areas of high temperatures and low humidity contaminating many kinds of food, showing affinity for grains and oilseeds [8,9,10]. The present study has been conducted to investigate the efficacy of electron beams for reducing aflatoxin B_1 in coconut agar residues.

2. MATERIAL AND METHODS

2.1. Samples

The strains of *Aspergillus flavus* were collected from the Mycotoxins Laboratory of ICB II-USP and were inoculated on to coconut agar medium and incubated for 7 days at 25 °C.

2.2. Irradiation

The samples were treated with Electron Accelerator, a Dynamitron Machine (Radiation Dynamics Co. model JOB, New York, USA), with 1.5 MeV energy, current of 7.1 mA, scan 100 cm and support speed 3.36 m min with a dose of 5.0 kGy and dose rate of 11.2 kGy s^{-1}. A control group (0 kGy) was analyzed to confirm the aflatoxin B_1 presence. A Red Perpex-batch HL (640 nm) dosimeter was used.

2.3. Extraction

Aliquot of 10 g of the sample was transferred to a beaker and 30 mL of chloroform was added. The mixture was macerated, filtered through filter paper and the chloroform extracts were dried and collected using a water bath. The dry extract was solubilized in 200 μL of chloroform and chromatographed as described by Lin and Dianese (1976) [11].

2.4. Determination of Aflatoxins

A thin layer chromatographic (TLC) method was used for qualitative estimation of aflatoxin B_1 [12].

2.5. Quantification of aflatoxins

The aflatoxins were quantified in a *Shimadzu Densitometer CS-9000* with a detection limit of 0.1 μg kg^{-1} in the Laboratory of Chemical and Natural Products of Pharmacology of Instituto Biológico, São Paulo.

2.6. Statistical analysis

All data were subjected to T test ($p < 0.05$) using *GraphPad Prism 5* software.

3. RESULTS AND DISCUSSION

In this study, the effect of electron beam irradiation was showed in Table 1. According to statistical analysis, significant difference was observed between the control sample (0 kGy) and the irradiated sample.

Table 1. **Effects of electron-beam irradiation in coconut agar residue.**

Samples	Control Group (0 kGy) μg kg^{-1}	Irradiated Group (5.0 kGy) μg kg^{-1}
1	26.40 [a]	7.93 [b]
2	36.11 [a]	8.76 [b]
3	27.79 [a]	7.18 [b]
4	33.84 [a]	7.31 [b]
5	36.92 [a]	8.60 [b]
6	22.38 [a]	8.08 [b]
7	41.51 [a]	8.99 [b]
8	38.69 [a]	7.79 [b]
Average	32.96 [a]	8.08 [b]
SD*	6.70	0.66

[a,b] Different superscript letters means significant difference ($p < 0.05$)

* Standard Deviation

The result of the quantification indicates that the treatment of coconut agar residues with electron beam irradiation destroyed 75.49 % of the aflatoxin B_1. Prado (2003) [13] observed a destruction of 69-74 % with doses of 15 kGy, 20 kGy, 25 kGy and 30 kGy in peanut. In a study carried out by Aziz and Youssef (2002) [14] it was observed a reduction of aflatoxin B_1 to not detectable levels in peanut, yellow corn, wheat and cotton seed meal, with a dose of 20 kGy, indicating that this dose was sufficient to destroy this toxin in these products. The irradiation of corn with a dose of 10 kGy was efficient to destroy aflatoxin B_1 and B_2 completely [15]. The irradiation with 4.0 kGy of chick-peas and groundnut seeds destroyed approximately 61 to 67 % of aflatoxin B_1, whereas application of 6.0 kGy results in a reduction of 74.3 to 76.7 % of aflatoxin B_1 [16]. Farag et al. (1995) [17] found that the gamma rays at 20 kGy were not effective in destroying completely the aflatoxins, reaching 83 % of reduction.

4. CONCLUSION

From above research results, the authors concluded that the electron beam treatment with 5.0 kGy was able to reduce the aflatoxin B_1 present in coconut agar medium.

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REFERENCES

1. M. A. Klinch, "*Aspergillus flavus*: The major producer of aflatoxin", *Mol. Plant Pathol.,* **8**, pp.713-722 (2007).
2. International Agency for Research on Cancer (IARC), *Aflatoxins: Natural occurring aflatoxins (Group 1), aflatoxin M_1 (Group 2B)*, IARC Scientific Publications, Lyon (1993).
3. R. H. Luchese, W. F. Harrigan, "Biosynthesis of aflatoxin: The role of nutritional factors", *J. Appl. Bacteriol.,* **74**, pp.5-14 (1993).
4. M. J. Sweeney, A. D. W. Dobson, "Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species", *Int. J. Food Microbiol.*, **43**, pp.141-158 (1998).
5. S. Aquino, "Efeitos da radiação gama no crescimento de *Aspergillus flavus* produtor de aflatoxinas e no emprego da técnica da reação em cadeia de polimerase (PCR) em amostras de grãos de milho inoculadas artificialmente", *Instituto de Pesquisas Energéticas e Nucleares*, São Paulo (2003).
6. A. Pak, E. Gonçalez, J. D. Felicio, M. M. Pinto, M. H. Rossi, I. C. Simoni, M. N. Lopes, "Inhibitory activity of compounds isolated from *Polymnia sonchifolia* on aflatoxin production by *Aspergillus flavus", Braz. J. Microbiol.,* **37**, pp.199-203 (2006).
7. C. Cavaliere, P. Foglia, C. Guarino, M. Nazzari, R. Samperi, A. Laganà, "Determination of aflatoxins in olive oil by liquid chromatography-tandem mass spectrometry", *Anal. Chim. Acta.,* **596**, pp.141-148 (2007).
8. Council for Agricultural Science and Technology (CAST), *Mycotoxins: Risks in Plant, Animal and Human Systems*, Task Force Report. Ames, USA (2003).
9. Y. Ito, S. W. Peterson, Q. T. Wicklow, T. Goto, "*Aspergillus pseudotamarii*, new aflatoxin producing species in *Aspergillus* section Flavi", *Mycol. Res.*, **105**, pp.233-239 (2001).
10. J. I. Pitt, "Toxigenic fungi: which are important?", *Med. Mycol.*, **38**, pp.17-22 (2000).
11. M. T. Lin, J. C. Dianese, "A coconut agar medium for rapid detection of aflatoxin production by *Aspergillus* spp", *Phytopathology,* **66**, pp.1466-1469 (1976).
12. L. M. V. Soares, D. B. Rodrigues-Amaya, "Survey of aflatoxins, ochratoxin A, zearalenone and sterigmatocystin in some Brazilian foods by using multi-toxin thin-layer chromatographic method", *J. Assoc. Off. Anal. Chem.,* **72**, pp.22-26 (1989).
13. G. Prado, E. P. Carvalho, M. S. Oliveira, J. G. C. Madeira, V. D. Morais, R. F. Correa, V. N. Cardoso, T. V. Soares, J. F. M. Silva, R. C. P. Gonçalez, "Effect of gamma irradiation on the inactivation of aflatoxin B_1 and fungal flora in peanut", *Braz. J. Microbiol.,* **34**, pp.138-140 (2003).
14. N. H. Aziz, B. M. Youssef, "Inactivation of naturally occurring of mycotoxins in some Egyptian foods and agricultural commodities by gamma-irradiation", *Egypt. J. Food. Sci.,* **30**, pp.167-177 (2002).
15. S. Aquino, F. Ferreira, D. H. B. Ribeiro, B. Corrêa, R. Greiner, A. L. C. H. Villavicencio, "Evaluation of viability of *Aspergillus flavus* and aflatoxins degradation in irradiated samples of maize", *Braz. J. Microbiol.*, **36**, pp.352-356 (2005).
16. N. H. Aziz, L. A. A., Moussa, "Reduction of fungi and mycotoxins formation in seeds by gamma irradiation", *J. Food Safety,* **24**, pp.109-127 (2004).
17. R. S. Farag, M. M. Rashed, A. A. Hussein, A. Abo-Hagar, "Effect of gamma radiation on the infected yellow corn and peanuts by *Aspergillus flavus", Chem. Mikrobiol. Technol. Lebensm.,* **17**, pp.93-98 (1995).