USE OF GAMMA RADIATION AND ELECTRON BEAM TREATMENT ON DECONTAMINATION OF COCONUT AGAR MEDIUM USED IN THE PRODUCTION OF AFLATOXINS

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Fungi of genera Aspergillus is largely distributed in the Brazilian ecosystem and known to produce aflatoxin, a natural contaminant in different food and feed commodities that represent a risk for consumers' health. To evaluate the ability to produce aflatoxins, that are thermoresistant metabolites of toxigenic moulds, the Aspergillus flavus strains are inoculate in coconut agar medium and after the extraction from agar, the residue obtained is placed in the autoclave bag as microbiological waste. The presented study evaluated fifty samples of waste coconut agar medium used in the mycotoxins laboratory to detect residual levels of aflatoxins (control group), submitted to gamma rays and to electron beam treatment in the doses of 25 and 50 kGy and compare the treatments on degradation of residual aflatoxins in the laboratory waste samples.

I. INTRODUCTION

Among the toxigenic moulds, *Aspergillus flavus* is the most frequently isolated and is known as aflatoxins producer, which are carcinogenic compounds. All isolates of *A. flavus* were screened for the ability to produce aflatoxins by the inoculation in coconut agar medium. There are a number of reports that show the toxigenic potential essay of *A. flavus* isolated in different products or foods [1, 2]. Lima et al.[3] reported that of the 19 isolates of *A. flavus* recovered in rice, 52.6% were aflatoxigenic, producing aflatoxins B_1 and B_2. Aquino et al.[4] demonstrated that the dose of 10kGy was effective on aflatoxin degradation in maize. The highly reactive free radicals can readily attack aflatoxins at the terminal furan ring, giving products of lower biological activity. The mutagenic activity of aflatoxin B_1 in an aqueous solution (5g μL^{-1} water) was reduced by 34%, 44%, 74% and 100% after exposure to gamma rays at 2.5; 5.0; 10.0 and 20.0kGy, respectively[5].

II. MATERIAL AND METHODS

II. A. Evaluation of toxigenic potential

Fifty samples of waste coconut agar were collected from Mycotoxins Laboratory of ICB II-USP. The strains of *Aspergillus flavus* were inoculated in coconut agar medium and incubated for 7 days at 25°C. Aliquot of 10g of this culture medium were transferred to a 200mL beaker containing 30mL of chloroform. The mixture was macerated, filtered through filter paper and the chloroform extracts were collected for dryness on a water bath. The dry extract was solubilized in chloroform and chromatographed as described by Lin and Dianese [6]. The waste samples were evaluated for the presence of aflatoxins and the fluorescence intensities of the spots formed were measured through comparison with aflatoxin standards under 365nm UV light. The method has a detection limit of 2 μg/kg[7].

II. B. Treatment by electron beam and gamma rays

The coconut agar sample was packed in polyethylene bag, containing 10g and it was treated by a ^{60}Co source Gammacell 220 (A.E.C. Ltda) with dose rate of 2.85kGy/h at doses of 0, 25.0 and 50.0kGy and using an Electron Accelerator, a Dynamitron Machine (Radiation Dynamics Co. model JOB, New York, USA), with 1.5MeV energy, current of 7.1mA, scan 100 cm and support speed 3.36m/min at doses of 0, 25.0 and 50.0kGy. CTA dosimeters for e-beam and Harwell Amber 3042 dosimeters to ^{60}Co were used for the measurement of radiation dose.

A control group of waste coconut agar was analyzed in parallel to confirm the aflatoxins presence in the residual medium, as described before.

III. RESULTS AND DISCUSSION

All control samples were positive to the presence of aflatoxins in coconut agar as a residual contaminant (in detectable levels above 2μg/kg). All irradiated samples treated with electron beam using the dose of 25kGy were positive to the presence of aflatoxins. Not detected levels were observed in 30% of samples treated with gamma processing at dose of 25kGy and the results of treatments using gamma rays and electron beam with the dose of 50kGy showed not detected levels of aflatoxins in all laboratory waste samples as demonstrated in TABLE I. The presence of water has an important role in the destruction of aflatoxin by ionizing radiation, since radiolysis of water leads to the formation of highly reactive free radicals[5]. The higher sensitivity of AFB_1 and AFB_2, respectively, to irradiation with dose of 50kGy compared to 25kGy, using electron beam and gamma rays, may be explained by the water are not available in a coconut medium agar, which explain the high doses for eliminating these mycotoxins or decreasing their concentrations to a not detectable level. There are no reports on the use of ionizing radiation in controlling of aflatoxins in laboratory waste. Ferreira-Castro et al.[8] reported that a high dose of 10kGy was not sufficient for a complete degradation of fumonisins in maize.

TABLE I. Qualitative results of detected and not detected levels of aflatoxins (2μg/kg of B_1 + B_2) in control and irradiated waste coconut agar samples.

SAMPLES	Control (kGy)	ELECTRON BEAM (kGy)		γ -RAY (kGy)	
	0	25	50	25	50
1	+	+	ND	+	ND
2	+	+	ND	+	ND
3	+	+	ND	+	ND
4	+	+	ND	+	ND
5	+	+	ND	+	ND
6	+	+	ND	+	ND
7	+	+	ND	+	ND
8	+	+	ND	ND	ND
9	+	+	ND	ND	ND
10	+	+	ND	ND	ND

C = Control group non irradiated (0kGy)
ND: Not detected levels (< 2μg/kg)
(+): Detected levels of aflatoxins (> 2μg/kg)

IV. CONCLUSIONS

From above research results, it came to the conclusion that the gamma ionizing radiation and the electron beam treatment were effective on degradation of aflatoxins in laboratory waste samples, after exposure to a dose of 50 kGy. Irradiation technology aimed at degradation of the aflatoxins residues found in laboratory waste agar resulting in less availability of the aflatoxins residues in environment.

ACKNOWLEDGMENTS

The authors would like to thank CNEN; IPEN-CNEN/SP; CAPES; CNPq; FAPESP and IAEA for financial support.

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