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EVALUATION OF FUNGAL CONTAMINATION IN IRRADIATED PHYTOTERAPIC

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ABSTRACT

Aspergillus spp. are widespread in nature. They are found in the micro-flora of air and soil and as contaminants of plant and animals. Some moulds are of particular interest from a public health viewpoint, because of their capability to produce mycotoxins. They are known as potent natural cancerogenic substances. The ability of ionizing radiation to kill microorganisms has been investigated since the late 19th century. There have many reports that radiation treatment is a suitable method for decontaminating food products and showed that the dose of 10 kGy of gamma radiation is sufficient to eliminate the actinomycetes from food and animal feed produtcs. The aim of this work is to determinate the effects of gamma radiation on fungi of phytotherapics used under the powder or teas forms.

1. INTRODUCTION

Moulds of the genera *Aspergillus* and *Penicillium* occur in different food and feed commodities several mycotoxins are known to be produced by *Aspergillus* and *Penicillium* species and many different Mycotoxins have been found as natural contaminants in different food (Aziz &Youssef,1991; Aziz,Refai,& Abd El-Aal,1990).The contamination of food and feed by these mycotoxins can lead to health problems in human and animals and can result in economic losses (Rodrick,1976). The purpose of this study was to isolate and identify the genera *Aspergillus* as a natural contaminant of medicinal plants in form of teas and powder and to estimate the occurrence of their mycotoxins and to study the effect of gamma irradiation on phytoterapics. The samples of phytotherapics purchased from pharmacies and street market in the five cities of São Paulo State. A total of 60 samples of four different phytotherapics denominated Boldo (*Peumus boldus* Molina), Espinheira Santa (*Maytenus ilicifolia* Martius), Guaraná (*Paulínia cupana*) and Sene *(Cassia angustifolia)*, were analyzed.

3. MATERIALS AND METHODS

3.1. Fungal Isolation and Enumeration

The samples of Boldo (*Peumus boldus* Molina), Espinheira Santa (*Maytenus ilicifolia* Martius) and Sene *(Cassia angustifolia)*, were prortioned in 10 g and were homogenized for 30 min in separated bottles containing 90 ml sterile distilled water. For fungal counts and identification, 0.1 ml of the dilutions, in a serial dilutions from 10^{-1} to 10^{-5} of the samples were seeded in duplicates and plated using the method in Dichloran 18% glycerol agar (DG 18). A total fungal concentrations were counted after five days at 27 °C. The isolation of Guaraná (*Paulínia cupana*) was perfomed by direct plating using DG 18 agar and for each particular treatment, thirty three grains in two replicate plates were prepared (eleven grains for each plate). The counting was made by frequency of fungi in grains, after five days at 27 °C.

3.2. Gamma Irradiation

The phytoterapics samples were irradiated in a polyethylene bags, each containig 10 g of medicinal plants, using a ^{60}Co gamma ray source (Gamma cell) located at Instituto de Pesquisas Energéticas (IPEN), in São Paulo city. The samples were exposed to doses of 0.0, 5.0 and 10.0 kGy. The gamma ray source gave a dose rate of 3 kGy/h.

3.3. Aflatoxin Analysis And Water Activity

Water activity (Aw) of the samples was determined in a AQUALAB CX-2 equipment from Decagon Devices and aflatoxin analysis were perfomed with Fifty grams (50 g) of each sample were extracted with methanol / 4% KCL (9+1). The extracts were clarified with 30% ammonium sulfate solution and then the aflatoxins were extracted by adding chloroform. Identification and quantification was conducted via thin layer chromatography by comparison with standards (Soares & Rodrigues-Amaya, 1989).

4. RESULTS AND DISCUSSION

Gamma radiation of foods has been proposed as a mean of food preservation. It becomes essential to practice proper post-harvest handling, drying and storage of commodities**.** Aziz *et al.* (1997) showed that 5 kGy was the dose required for complete elimination of actinomycete contaminated cinnamon and peppermint. It may be the conclued that 5 kGy is the lethal dose for most fungi and actinomycete contaminated medicinal plant samples investigated. In this work, the data reveal that the number of unit forming colonies per gram decrease with increasing dose. The data obtained indicated that the percentages of total fungal florae in Guaraná grains in the control samples was 100% of *Rhizopus* spp. (Table 2) and almost all samples of Boldo (*Peumus boldus* Molina), Espinheira Santa (*Maytenus ilicifolia* Martius)

and Sene *(Cassia angustifolia)*, examined in this study were contaminated with *Aspergillus* spp., but they did not detect aflatoxins in any sample. It may be explained by low water activity of samples (Table 3). The presence of water has an important role in the fungal toxin production. The minimal *Aw* for mycotoxin production was determined to be around 0.82 at 25 – 37 °C (Smith, 1993). It was observed that the dose of 5 kGy was effective to reduce the contamination of samples. When phytoterapics samples were irradiated at dose level 5 kGy, the total fungal counts were reduced to 0 and 0.2 x 10^2 per gram in Boldo and Sene (Table 1). Meanwhile, the dose of 10 kGy was more efficient to decontamination, how observed in all irradiated samples. It may be conclued that 10 kGy is the lethal dose for the most fungi, comparing with control samples, in four different phytoterapics investigated. The efficacy of gamma radiation for decontamination of certain spices has been already reported and the authors revealed that exposure to gamma radiation in the dose range of 7.5 – 10 kGy decontaminated adequately all investigated spices (Farkas, 1983; Sharma *et al.,* 1984) and this result is in accordance with our results.

Table 1. Number of colonies forming units per gram (CFU/g) of *Aspergillus* spp. in irradiated phytoterapics.

Samples	Dose		
	0 kGy	5 kGy	10 kGy
Boldo*	1,6 x 10^2	0,2 x 10^2	0
Espinheira Santa*	7,7 x 10^2	0	0
Sene*	18,8 x 10^2	0,2 x 10^2	0

*Average of five replicates

Table 2. Frequency of of *Rhizopus* spp. in grains of Guaraná.

Samples of 33 grains*	Dose		
	Control	5 kGy	10 kGy
Frequency	100 %	2.45 %	0 %

*Average of five replicates

Table 3. Average of water activity of samples.

Samples	Aw
Sene*	0.52
Boldo*	0.54
Espinheira Santa*	0.50
Guaraná*	0.61

*Average of five replicates

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