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INHIBITION OF AFLATOXIN PRODUTION BY ASPERGILLUS FLAVUS USING LOW LEVEL γ - IRRADIATION

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SUMMERY

Effects of selected low level doses of γ - Radiation (100-400 K rad) on the ability of toxin strain of Asperg illus flavus to survive and produce aflatoxin in culture medium and pistachio nuts have been studied. A reduction of 60 per cent in growth and spore production by Aspergillus flavus in culture medium was observed after treatment with 100 K rad of γ - Radiation.

In spore inoculated pistachio nuts, 100 K rad of γ - Radiation reduced the aflatoxin B_1 and G_1 production by 75% after eight weeks storage period. The aflatoxin production ability by Aspergillus flavus on pistachio nuts was affectively eliminated by the treatment of spore inoculated pistachio nuts with 200 K rad of γ-Radiation,although very little growth could be detected after eight weeks' storage of 400 K rad γ-irradiated pistachio nuts.

INTRODUCTION

Aflatoxins are secondary metabolites produced under specific conditions by certain strains of moulds called

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Aspergillus flavus and Aspergillus parociticus. They are found as human food contaminants in many parts of the world (1) and recently in the south eastern region of Iran. Aflatoxins are both toxic and carcinogenic to a wide range of animals(2). One of these metabolites, Aflatoxin B_1, is the most potent carcinogen of liver known for rat (3). It has been associated with high liver cancer incidence in certain parts of Africa (4).

The use of low level γ-Radiation to extend the storage life of certain foods has been studied by numerous workers (5,6). These levels of irradiation do not sterilise food products but rather reduce microbial populations and thereby extend storage life. This application of γ-Radiation was also employed to extend the shelf life of fresh bread used in the food system for manned space flight missions (7,8).

This work was initiated to study the effects of selected low level doses of γ-Radiation on the ability of the strain of Aspergillus flavus to survive, grow and produce aflatoxin in culture medium, and pistachio nuts.

MATERIALS AND METHODS

Aspergillus flavus strain M216 used in this study,was obtained from food and drug administration department of U.S.A. This is a very potent strain for production of aflatoxin. Stock culture was maintained at 2-5^{o}C on slant of Difco potato dextrose agar(Difco Laboratories,Detroit, Michigan, U.S.A.) in screw cap test tubes.

INOCULATION

Culture of the toxic mould was grown on corn meal agar for 5-6 days at 25^{o}C nutil well sporulated. The spore were

washed from the slant with a 1% solution of Triton X100. The suspensions were quantified using a haemocytometer and diluted to obtain 10^6 spores per ml of suspension.

Pistachio nuts used in this study were originally tested for presence of aflatoxin and mould. Three kg of nuts were placed inside a bacteriological glove box and 30 ml of inoculum was spread and mixed. Hundred gram of inoculated pistachio nuts were packaged in polyethylene bags and sealed. They were then irradiated by triplicates at doses of 0.0,100,200,300 and 400 K rad at 25°C using a Co-60 source and placed in a glass jar containing water in the lower compartment and Kept at 28°C with 95% relative humidity. After 4,6 & 8 weeks they were examined for visible mould growth and aflatoxin production using pon's method(9). The concentration of aflatoxin B_1 and G_1 in extract solution was estimated quantitatively by visual comparison of fluorescence of sample to that of known standards on a silica gel G type 60 T.L.C. plate. The T.L.C. development solvent system used was comprised of chloroform-acetone-acetic acid and water(90-10-2-2 v/v) which was devised in our laboratory. Aflatxin B_1 and G_1 standards were obtained from Macor Chemicals,Jerusalem, Israel.

Young culture of Aspergillus flavus strain M216 was placed on corn meal agar and grown for 24 hrs. Then it was exposed to 0.0, 100,200,300 and 400 K rad doses of γ-Radiation and allowed to grow for 10 days at 25°C. Analyses were carried out by visual inspection,measuring diameter of growth on the surface of plate and spore counting.

The gas liquid chromatography analysis of hexane extract of pistachio nuts treated with 400 K rad of γ- Ra-

diation was carried out on a Varian Model 1700 using a 15% polyethylene glycol succinate on a Chromosorb W column of ¼ in.i.d.x 6 ft.long using a flame ionzation detector.

RESULTS

The amount of growth and spore production of Aspergillus flavus strain M216 in corn meal agar after γ-Radiation doses of 0.0 to 400 K rads which was stored for 10 days was brought about by measuring the radius of mould growth and spore counting by haemocytometer is shown in Tables 1 and 2. Table 3 shows quantitatively the aflatoxin production ability of spore inoculated pistachio nuts treated whith 0.0 to 400 K rads of γ-Radiation and stored for 4,6 and 8 weeks. The amount of growth of Aspergillus flavus on γ-irradiated pistachio nuts which were stored for 8 weeks and assessed visually is given in Table 4.

Gas liquid chomatography of hexane extract of 400 K rad-irradiated pistachio nuts shows no signs of long chain hydrocarbon production.

DISCUSSION

The results clearly indicate that 200 K rad dose of γ-Radiation completely eliminates the growth and spore production ability of Aspergillus flavus strain M216 in corn meal agar after storing at 28°C for 10 days.

A 60% reduction of growth and spore production ability is brought about by 100 K rad of γ-radiation undér the same conditions. The observed results are partially due to degradation of nutrient and breakdown of culture medium by ionising radiation.

TABLE 1

Effect of γ-irradiation on radius of growth of A. flavus M216 on corn meal agar stored at 28°C for 10 days.

Sample	Control 0.0 (cm)	100 Krad	200 Krad	300 Krad	400 Krad
1	3.1	1.2	-	-	-
2	3.2	1.2	-	-	-
3	3.2	1.1	-	-	-
4	3.2	1.1	-	-	-
5	3.1	1.1	-	-	-
Mean valus	3.16	1.14	-	-	-

TAPLE 3

Effect of γ-irradiation on A. flavus ability for aflatoxin B_1 and C_1 production on pistachio nuts at different storage pariods.

Storage time (weeks)	Control 0.0		100 K rad		200 K rad		300 K rad		400 K rad	
	B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1
4	400	200	80	40	-	-	-	-	-	-
6	666	382	151	78	-	-	-	-	-	-
8	421	521	230	128	-	-	-	-	-	-
	Total aflatoxin production									
	B_1 and G_1				B_1 and G_1					
4	600				120	-	-	-	-	-
6	1048				259	-	-	-	-	-
8	1442				358	-	-	-	-	-

TABLE 2

Effect of γ-irradiation on total number of spores produced by A. grown pistachio nu-- corn meal agar stored at 28°C for 10 days.

Sample	Dose 0.0	100 K rad	200 K rad	300 K rad	400 K rad
1	155×10^6	53×10^6	-	-	-
2	158×10^6	55×10^6	-	-	-
3	156×10^6	55×10^6	-	-	-
4	153×10^6	54×10^6	-	-	-
5	-	56×10^6	-	-	-
Mean value	154×10^6	54.6×10^6	-	-	-

TABLE 4

Effect of γ-irradiation on growth of Aspergillus flavus strain M216 on pistachio nuts after 8 weeks' storage,estimated by visual observation (+ is a criterion of measurement).

Dose (K rad)	Control 0.0	100 K rad	200 K rad	300 K rad	400 K rad
Visual gowth	25+	5 +	3 +	2 +	1 +

In the case of spore inoculated pistachio nuts treated with doses of 0.0 to 400 K rad of γ-radiation, the results(Table 3) indicate that 100 K rad dose of γ-radiation reduces the aflatoxin production ability of Asperg - illus flavus about 75% after eight weeks' storage at 28°C and RH of 95%. The aflatoxin production ability by Aspergillus flavus was completely eliminated after treatment with 200 K rad dose of γ-radiation and storage for eight weeks at 28°C and RH of 95%.

Although the aflatoxin production ability of Aspergillus flavus is completely ceased after treatment with 200 K rad dose, the mould ability to growon pistachio nuts followed a different pattern to that of culture medium

Since in the case of 400 K rad treated pistachio nuts there are still signs of growth after eight weeks' storage period. (Table 4) This obervation may be due to the fact that pistachio nuts are more resistant to radiation damage than corn meal agar culture medium.

Gas liquid chromatography of hexane extract of 400 K rad γ-irradiated pistachio nuts show no signs of long chain hydrocarbon production, whilst its presence would have been indicative of breakdown of the lipid content of the nuts. (10).

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