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Producibility of Aflatoxin by *Aspergillus parasiticus* in Barley and Their Radiosensitivity

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*Aspergillus parasiticus*에 의한 보리의 Aflatoxin 生成과 감마線의 影響

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Abstract: The effect of gamma irradiation on production and accumulation of aflatoxin on natural substrate (barley) by *Aspergillus parasiticus* NRRL 2999 has been studied in some detail. Gamma irradiation at five doses, 0, 50, 100, 200 and 400 Krad was applied to the grain either soon after moisture equilibration (3 days after inoculation) or 10 days later (13 days after inoculation). And the results were as in the followings.

1. Increase in moisture content from 17% to 25% greatly increased the aflatoxin concentration, especially at zero irradiation dose. 2. Prolongation of the incubation period prior to irradiation from 3 to 13 days resulted in greater accumulation of aflatoxin. 3. Two hundreds Krad applied 13 days after inoculation on barley stored at 25% moisture (100% RH) and 25°C led to higher aflatoxin production than 100 Krad or even 50 Krad. 4. The relative proportion of the principal aflatoxins in relation to irradiation showed that aflatoxin G was elaborated at a significantly higher rate than aflatoxin B.

Introduction

Since the discovery in the early 1960s of aflatoxins, a series of highly potent carcinogens produced by *Aspergillus flavus*, research has focused new attention on the problem. The production of aflatoxin for experimental studies has been investigated on a variety of agricultural substrates and conventional microbiological media.

Several approaches have been investigated for detoxification of aflatoxin-contaminated foods and feeds including physical or mechanical, chemical, and biological methods. One of them is the ionizing radiation for microorganisms and insects which cause food spoilage and deterioration (Bellamy, 1959; Niven, 1958; Urbain, 1978). Stored foods, mainly grains, are subject to both insect infestation and fungal infection. Irradiation has been considered as a means of protecting stored commodities from these hazards. Among the storage fungi, *Aspergillus flavus* and *Asp. parasiticus* may produce potent hepatotoxic and carcinogenic substances, known as aflatoxin, on many foods, such as peanuts, corn, rice wheat, potatoes and sorghum (Detroy *et al.*, 1971; Diener and Davis, 1969; Schindler *et al.*, 1980).

Irradiation has been shown to destroy the conidia of *Asp. flavus* (Padwal-Desai *et al.*, 1976a, b). It is

possible, however, that irradiate foods may become more susceptible to subsequent infection by aflatoxin-producing fungi (Pryyadarshini and Tulpule, 1976), or that these fungi may be stimulated to produce more toxin after they have been exposed to certain levels of gamma radiation (Applegate and Chipley, 1973, 1974; Jemmali and Guilbot, 1970). According to one report, wheat irradiated for insect disinfestation must be subsequently stored for at least three months before elaboration, if it occurs, may proceed differently than in the absence of irradiation.

The purpose of this investigation was to study the effect of gamma rays on the production of aflatoxin in barley inoculated with conidia of *Aspergillus parasiticus* before irradiation.

Materials and Methods

Organims. A culture of *Aspergillus parasiticus* NRRL 2999 obtained from the Nothern Regional Research Lab., Peoria, IL, was grown on potato-dextrose-agar(Difco Inc.), at 25°C for 9 days. The conidia were harvested with sterilized, distilled water containing 0.01% tween 80 passed through 16-fold cheese cloth, washed in water by centrifugation and finally suspended in water at the concentration of about 10^6 spores (conidia) per ml.

Preparation of barley samples. Barley used in this study was a Coho spring barley which received from the Department of Soil and Crop Science, Michigan State University.

Barley were surface-sterilized by washing for 2 minutes in a 1% sodium hypochlorite solution rinsed twice in sterile distilled water and dried to about 12% moisture content. Fifty grams of sterilized barley were placed in 4 oz square bottles (4×4×8cm), and the moisture content was adjusted to 17, 20 and 25% by adding the required amount of sterile distilled water and 1ml of spore suspension. The bottle then was closed, the grain was shaken mechanically for 3 min, and placed in a refrigerator 3 days; during this time they were occasionally shaken in order to assure uniform distribution of moisture throughout the sample.

Gamma-irradiation. The bottles of barley were exposed to gamma irradiation from a 50.00 Curie ^{60}Co source immediately after moisture equilibration, and the other half after 10 days of incubation at 25°C. Five radiation levels were used: 0, 50, 100, 200 and 400 Krad. Fricke dosimetry was used for calibration.

Culture. After irradiation, as well as during the 10 days pre-irradiation period, the bottles were stored at 25°C in glass vessels (converted desiccators) of 3 different constant relative humidities, namely 85%, 92% and 100%, which were obtained by using saturated solution of KCl,saturated solution of $NH_4H_2PO_4$ and water, respectively. (Wink and Sears, 1950; Hubbard *et al.*, 1957; Houston, 1952). The mouths of bottles were left loosely closed to allow free exchanged of air, and sample were tested after 60 days. That these solutions actually did maintain the moisture contents when samples tested during storage, within the range of experimental error(about plus or minus 0.3%). Moisture content was measured by the two-stage oven method recommended by the AACC approved methods (1971).

Aflatoxin determination. Molded barley (25g) was ground in Waring blender for 3 min and extracted with water (25ml), diatomaceous (Hyflo cel, 25 g) and chloroform (250ml) on a shaker for 30min. The thin-layer chromatography method procedures described by the AOAC methods (1980) were used. Aflatoxin was determined quantitatively in each extract by spotting samples onto thin-layer glass (precoated Silica Gel-HR 25, Brinkman Ins., Inc.). The plates were developed with ether-methanol-water solvent system (96:3:1, v/v) at 23 to 25°C until aflatoxins reach R*f* 0.3 to 0.7. Amounts of aflatoxin present were determined by a double-beam scanning-recording-integrating spectrodensitometer (Model SD 3000-4. Schoeffel Instr., Inc.). To confirm the presence of aflatoxin, standards and extracts were spotted and developed with the upper phase of bezene-ethanol-water (46:35:19, v/v) solvent system (Waltking *et al.*, 1973). The combined extracts of 3 bottles were used for the thin-layer chromatography and the plates were scanned twice.

Results and Discussion

The aflatoxin production in the barley at different radiation level and various moisture content of post-irradiation storage are presented in Tables I and II, Figures 1 and 2.

Table I(four aflatoxins) and Figure 1 (total aflatoxins) show the aflatoxin levels in barley irradiated soon after moisture equilibration(3 days after inoculation of *Asp. parasiticus*). The four major aflatoxins production by *Asp. parasiticus* in barley was greatly reduced by gamma irradiation.

It is striking that the irradiated barley invariably showed lower aflatoxin levels than in the corresponding controls. It was observed earlier that even at the disinfestation dose there was reduction in the microbial count(Rao *et al.*, 1976). However,yet although the levels of aflatoxin in these samples were low, the amounts produced at this moisture level indicates a potenial danger of aflatoxin production.

There was no stimulation of aflatoxin production observed in the barley inoculated with NRRL 2999. Noteworthy is the pattern of aflatoxin accumulation in relative amounts of the B_1 and G_1 components. The relative proportion of the principal aflatoxins in relation to incubation period showed that aflatoxin G was elaborated at a significantly higher rate than the B although amounts of aflatoxin accumulated in irradiated samples were less than zero irradiation dose.

From these data, it was observed to optimal moisture content(or RH) for maximal biogenesis of the toxin, *i.e.*, increase in moisture content from 17% to 25% greatly increased the aflatoxin concentration.

Table II and Figure 2 show the aflatoxin levels in barley irradiated 10 days after moisture equilibration (13 days after inoculation). Gamma irradiation of the barley inoculated with *Asp. parasiticus* inhibited aflatoxin production. At the 200 Krad treatment, aflatoxin production was reduced by 32% from the nonirradiated control but unexpectedly, aflatoxins production was nearly 2 times more than 100 Krad even 50 Krad. Jemmali and Guilbot(1970) reported that gamma irradiation dose below 200 Krad may induce or

Table I. Effect of irradiation on aflatoxin production in barley. Irradiation 3 days after inoculation with *Aspergillus parasiticus* NRRL 2999. Post-irradiation storage at 25°C and 60 days.

Moisture content (%)	Dose(Krad)	Aflatoxin, μg per kg				Ratio(B_1=1.00)		
		B_1	B_2	G_1	G_2	B_2	G_1	G_2
17	0	7	1	32	4	0.14	4.57	0.57
	50	4	1	9	1	0.25	2.25	0.25
	100	1	ND*	2	1	—	2.00	1.00
	200	TR**	ND	TR	ND	—	—	—
	400	TR	ND	TR	ND	—	—	—
20	0	65	10	506	107	0.15	7.78	1.65
	50	8	2	17	1	0.25	2.13	0.13
	100	6	1	11	1	0.17	1.83	0.17
	200	4	ND	8	1	—	2.00	0.25
	400	2	ND	7	1	—	3.50	0.50
25	0	4537	358	18793	2465	0.08	4.14	0.54
	50	757	89	3610	113	0.12	4.77	0.15
	100	94	17	553	78	0.18	5.88	0.83
	200	89	8	492	60	0.09	5.53	0.67
	400	12	2	50	10	0.17	4.17	0.83

*ND: not detected. **TR:trace(less than 1 μg per kg).

Table II. Effect of irradiation on aflatoxin production in barley. Irradiation 13 days after inoculation with *Aspergillus parasiticus* NRRL 2999. Post-irradiation storage at 25°C and 60 days.

Moisture content(%)	Dose(Krad)	Aflatoxin, μg per kg				Ratio(B_1=1.00)		
		B_1	B_2	G_1	G_2	B_2	G_1	G_2
17	0	11	1	44	6	0.09	4.00	0.55
	50	5	ND*	9	1	—	1.80	0.20
	100	2	ND	3	1	—	1.50	0.50
	200	2	ND	4	1	—	2.00	0.50
	400	ND	ND	2	ND	—	—	—
20	0	182	29	1,117	293	0.16	6.14	1.61
	50	10	1	92	20	0.10	9.20	2.00
	100	10	3	88	17	0.30	8.80	1.70
	200	7	1	38	9	0.14	5.43	1.29
	400	2	ND	9	1	—	4.50	0.50
25	0	9,354	1,350	36,997	9,299	0.14	3.96	0.99
	50	1,780	226	7,363	1,324	0.13	4.14	0.74
	100	1,691	195	7,382	1,229	0.12	4.37	0.73
	200	3,159	387	11,904	2,608	0.12	3.77	0.83
	400	931	63	4,565	644	0.08	4.90	0.69

ND:not detected.

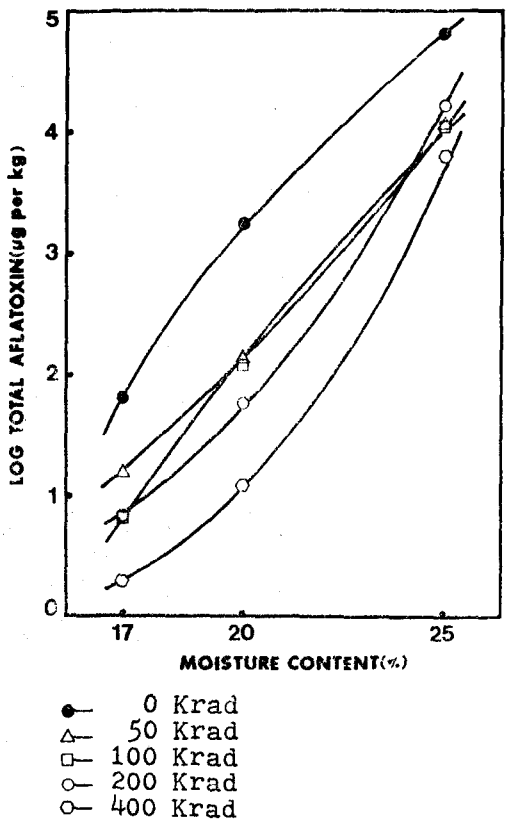

Fig. 1. Effect of moisture on the total aflatoxin contents in barley irradiated at 0~400 Krad after 10 days of incubation.

increase aflatoxin production in *Asp. flavus*, when grown on Czapek's broth fortified with yeast extract. Applegate and Chipley (1973) observed increased aflatoxin B_1 production by *Asp. flavus* at 150, 200 and

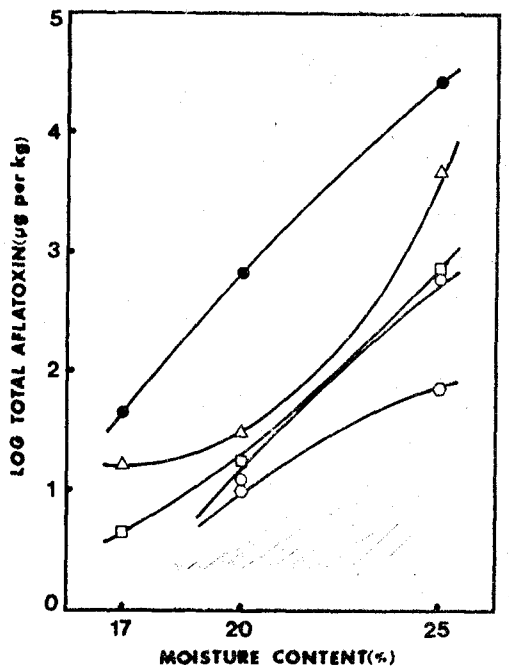

Fig. 2. Effect of moisture on the total aflatoxin contents in barley irradiated at 0~400 Krad immediately after inoculation.

300 Krad of irradiation when the organism was grown on a cracked wheat substrate. *Asp. flavus* and *Asp. parasiticus* are generally recognized as morphologically distinct, few physiological differences are known (van Walbeek, 1973).

This observation must be considered of great importance to public health. However, the reason for the increased production of toxin by fungus grown on irradiated foods is not clear. One possibility may be considered. Irradiation is known to produce certain biochemical changes in the food and these may result in an increased somatic growth of the fungus and hence in an increase in aflatoxin production. These biochemical changes could also increase the availability of precursors for toxin production without necessarily affecting the somatic growth of the fungus. Therefore, if irradiated foods become infected or infected foods with mycotoxin producing mold during storage, as is possible, particularly if conditions of storage are not satisfactory and the moisture content increases, the risk of greater amounts of toxin being formed must be considered as being very real.

摘 要

Aspergillus parasiticus NRRL 2999에 의한 보리의 aflatoxin生成에 미치는 코발트-60감마線의 影響을 調査하였다. 감마線은 平衡水分된 後(接種 3日後), 그리고 10日동안 培養後 0, 50, 100, 200 및 400Krad로서 照射하였으며 그 結果는 다음과 같다.

1. 水分含量이 17%에서 25%로 增加함으로써 aflatoxin의 生成量이 顯著하게 增加하였으며, 특히 無照射區에서 크게 增加하였다.

2. 培養期間을 3日에서 13日로 延長하여 照射함으로써 aflatoxin의 生成이 더욱 增加하였다.

3. 接種 13日後 200Krad로 照射하여 25°C에서 培養된 25%水分含量(RH:100%)에서는 50 또는 100Krad로 照射된 것보다 aflatoxin의 生成이 增加하였다.

4. Aflatoxin의 種類別 蓄積比率은 aflatoxin G가 B보다 顯著하게 大量으로 蓄積되었다.

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