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AFLATOXINS IN INOCULATED RAW SESAME SEEDS AND ITS PROCESSED SESAME HALVA

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Abstract

Raw sesame seeds were examined for the incidence of fungi producing ***aflatoxins***, and ***aflatoxin*** control in the processed sesame halva.

The incidence of fungal flora in raw sesame seeds were high for ***Penicillium*** (40%), followed by 25% for ***Aspergilus niger*** and 35% fortwospeciesof ***Aspergillus flavus*** producing ***aflatoxins***.

Raw sesame seeds were subjected to the following treatments to control fungal growth and aflatoxin production: 1) Irradiation at 10 KGy, 2) Addition of 1.5% propionic acid and 3) Irradiation and addition of propionic acid. The treated sesame, seeds were compared with the inoculated control sample (without treatment)for the amount of, aflatoxins produced when the seeds were inoculated and incubated with the mixed fungal culture of A. parasiticus NRRL 2999 and ***A. flavus*** EMCC 274.

Irradiation of sesame seeds at 10 KGy before inoculation with 1 ml (105 spores)/gof the mixed fungal culture increased the productionof ***aflatoxins***. This observation might be due to the destructive effect of irradiation on the competitive organisms present in sesame seeds, so improved growth and activity of the inoculated fungal culture. However, addition of 1.5% propionic acid to the irradiated seeds, reduced the stimulatory effect of irradiation on the production of ***aflatoxins***.

The processing of sesame halva from the sesame seeds contaminated with ***Aspergillus*** strains showed a significant reduction in aflatoxins. The highest decrease in ***aflatoxin*** B1 (90%) occurred in sesame halva processed from the irradiated seeds and the addition of propionic acid at 1.5% level.

INTRODUCTION

Mycotoxins can reach human or animal foods either through direct or indirect contamination and cause mycotoxicosis by ingestion. In direct contamination, the food materials support the toxigenic mold growth. Almost, all foods would be susceptible to mold growth at some stages during their production, processing, transportation an storage. By contrast, indirect contamination would occur when a food ingredient is contaminated with mycotoxin (Jarvis, 1976). Since the recognition of the hazards of ***afla-***

toxin contamination of food and feed commodities, survey has been conducted on the incidence of ***aflatoxins*** in food and feed materials. The Food and Agricultural Organization (FAO) reported that 25% of the world's food crops are affected by mycotoxins (Mannon and Johnson, 1985).

Burzynska (1971) isolated 124 strains of ***Aspergillus, Penicillium, Mucor, Trichothecium*** and ***Cladosporium spp.***, from 74 samples of foods imported into Poland. The samples were peanuts, walnuts, hazelnuts, cocoa beans, cocoa, figs, rice, rye and sesame seeds. He added, Aspergillus flavus was the only strain to produce aflatoxins. Moreover, Sengupta and Roy (1983) found aflatoxin B1 in raw peanut oil (0.01-0.35 ppm) and in raw sesame oil (0.02-0.15 ppm). Jonsyn (1988), isolated three toxigenic Aspergillus spp. from sesame seeds sampled from 4 locations in Sierraleone. The isolated Asp.flavus produced only aflatoxin B' and G1.

This study aimed to investigate the presence of fungal flora and their ability to produce aflatoxins; the treatment of sesame seeds with propionic acid or radiation to control aflatoxins in the processed sesame halva.

MATERIALS AND METHODS

1- Materials:

1-1- Three kilograms of raw sesame seeds were obtained from the International Food Industry Company (Sweet Food), 10th Ramadan City, Egypt.

1-2- Two active strains of ***Aspergillus flavus*** EMCC 274 and ***Aspergillus parasiticus*** NRRL 2999 were obtained from Cairo Microbiological Resource Center (CAIM), Ain Shams University, Egypt.

1-3-a) Medium of yeast-extract sucrose medium (YES) (Davis *et al.*, 1966) was used for the growth, isolation and identification of the ***fungal flora***.

b) Medium of potato-dextrose agar (PDA) (Oxoid, 1982) was used for the detection of the ability of the isolated fungi to produce ***aflatoxins***.

c) Medium of malt-salt agar (Aziz and Bean, 1995) was used for the isolation and identification of the fungi producing ***aflatoxins***.

1-4- Propionic acid was obtained from El-Nasr Pharmaceutical Chemicals Co., Cairo. It was used at concentration of 1.5% (Aziz, 1990).

1-5- Pure aflatoxins B_1, B_2, G_1 and G_2 were obtained from Sigma Chemicals Company, St. Louis, U.S.A. They were used as standards for ***aflatoxin*** determinations.

1-6- Ready made plates for thin layer chromatography (TLC) aluminium sheets with 0.2 mm thickness of silica gel 60 were obtained from E. Merck, Darmstadt, West Germany.

2- Methods:

2-1- Isolation and purification of the ***fungal flora*** present on the raw sesame seed samples was done according to the method of Quasem and Christensen (1958).

2-2- The purified ***fungi*** were identified according to Barrnett and Hunter (1972) using the facilities of Mycotoxin Lab., National Research Center, Giza - Egypt.

2-3- The fungal isolates were examined for ***aflafoxin*** production according to the method of Bothast and Fennell (1974).

2-4- Spore suspensions of each ***fungal*** strain of ***A. flavus*** and A. ***parasificus*** were prepared according to the method of Farag *et al.*,(1986a).Each suspension was adjusted to contain approximately 10^5 spores/ml for both strains.

2-5- Sesame seeds (2 kg) were divided into two equal portions:

a) An unirradiated portion (control) (S_1).

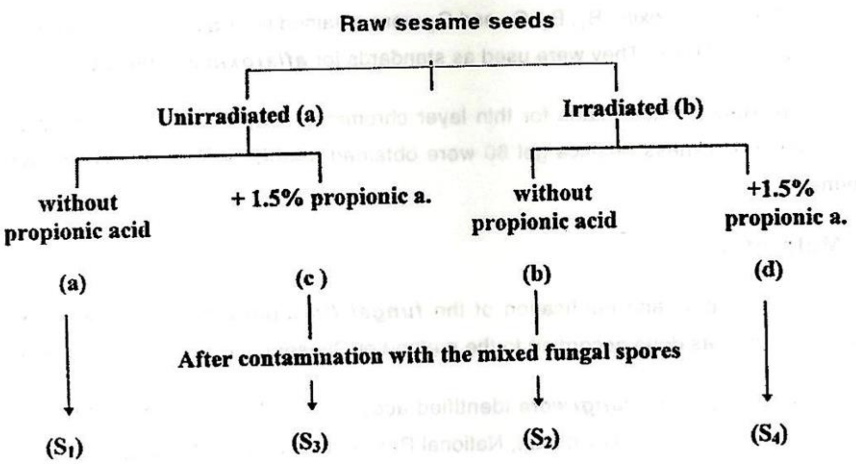
b) A protion irradiated (S_2) at 10 Kgy at theNationalCenterforRadiation Research and Technology, Nasr City, Cairo. The used Irradiator was Mega Gamma-I model "AECLJS 6500".

1.5% propionic acid was added to half of each portion hence:

c) Sesame seeds with addition of propionic acid (S_3).

d) Sesame seeds (irradiated) with addition of 1.5% propionic acid, (S_4).

The different sesame seed treatments are illustrated by the following scheme:



Each sesame treatment was inoculated with the spore suspension of amixed culture of both ***A. flavus*** EMCC 274 and ***A. parasiticus*** NRRL 2999 at a rate of 1 ml (105 spore)/g of sesame seed samples and incubated at 25°C for 15 days. Then, ***aflatoxins*** were determined in each of the four treatments, following the metho of Schuller and Van Egmond (1983).

The four sesame seed treatment samples were used to make sesame tahina by the continuous crushing method. Then, sesame halva was processed using each of the aforementioned sesame tahina and ingredients as mentioned by El-Dokany (1965). Treatments were carried out in triplicates. ***Aflatoxins*** were determined in the sesame halva samples from each treatment following the method of Schuller and Van Egmond (1983).

RESULTS AND DISCUSSION

Incidence of fungi on raw sesame seeds and their ability to produce *aflatoxins*:

The results in Table 1 show the average of occurrence of different ***fungal species*** isolated from sesame seed samples. It could be pointed out that ***Aspergillus species*** in raw sesame seeds consisted of ***A. niger, A. flavus*** (Light green, Lg) and ***A.flavus*** (Brown, Br) in decreasing order, i.e. 25, 20 and 15%, respectively. It is worth to mention that ***Penicillium spp.*** was found at a high level of 40%.

Regarding the ***fungal species*** isolated from sesame seed samples, it is obvious that ***A. flavus*** (Br) and ***A. flavus*** (Lg) are the two species capable of producing ***aflatoxins*** (Table 1). These results are in accordance with those reported by Jonsyn (1988) who isolated three toxigenic ***Aspergillus spp.***, from sesame seed samples, and their ability to produce ***aflatoxins***.

Aflatoxin control in sesame seeds and the processed sesame halva:

The results of ***aflatoxin*** production by the mixed fungal culture of *A. pararisitus* NRRL 2999 and ***A. flavus*** EMCC 274 inoculated on the different treatments of sesame seeds are presented in Table 2 and illustrated in Fig. (1&2). Irradiation of sesame seeds (S2) at 10 Kgy before inoculation with 1m 1 (105spore)/g of the mixed fungal culture increased the production of ***aflatoxins*** B_1, B_2, G_1 and G_2 by 16.3, 6.7, 7.7 and 56.3%, respectively after incubation at 25°C for 2 weeks. This in crease in the production of ***aflatoxins*** in irradiated sesame seeds might be due to the destructive effect of irradiation for the competitive organisms present in sesame seeds, so improved growth and activity of the inoculated mixed fungal culture. These results are in agreement with those obtained by Schindler ***et al.***, (1980) who reported that ***aflatoxin*** B1 productionby ***A. flavus*** M- 141, on sterile rice substrate, increased as the irradiation dose increased from 0.16 to 4.75 Kgy. Moreover, Abd El-Aal and El-Bazza (1990) reported that ***aflatoxins*** production by A. flavus spore suspension exposed to gamma radiation were higher than non-treated spore suspension at a dose level of 1.5 and 2.0 KGy.

Addition of 1.5% propionic acid to sesame seeds before inoculation (S3) with the mixed fungal culture reduced the production of ***aflatoxins*** B_1, B_2, G_1 and G_2 by 37.2, 89.9, 92.3 and 56.3%, respectively. Addition of 1.5% propionic acid to irradiated sesame seeds (S_4), also reduced the stimulatory effect of irradiation to the production of ***aflatoxins*** B_1, B_2, G_1 and G_2 by 20, 10, 22.9 and 23.2%, respectively.

The processing of sesame halva from the contaminated sesame seeds with the strains of ***Aspergillus*** showed a reduction effect on the content of ***aflatoxin*** concentrations in the final product. Results are shown in Table 5 and illustrated by Fig. 2, these results indicated that using unirradiated sesame seeds in sesame halva processing decreased I the level of ***aflatoxins*** B_1, B_2, G_1 and G_2 in the produced sesame halva (SH_1) by 12.9, 6.7, 6.2 and 62.5%, respectively. Moreover, using irradiated sesame seeds in processing sesame halva increased the degradation of ***aflatoxins*** B_1, B_2, G_1 and in the Produced sesame halva (SH_2) by 6, 3.75, 5.1 and 24 %, respectively. Utilization of unirradiated sesame seeds with the addition of 1.5% propionic acid in the manufacture of sesame halva decreased the level of ***aflafoxins*** B_1, B_2, G_1 and G_2 in the produced sesame halva (SH_3) by 13.3, 34.2, 7.2 and 54.3% respectively.

It is worth to mention that using sesame tahina from irradiated sesame seeds with the addition of 1.5% propionic acid in the manufacture of sesame halva reduced the concentrations of ***aflatoxins*** B_1, B_2, G_1 and G_2 in the produced sesame halva (SH_4) by 90, 8.3, 22.2 and 47.9%, respectively.

Therefore, it could be concluded that the highest significant degradation in ***aflatoxins*** concentration, as a result of sesame halva processing, occurred in the treatment of irradiated seeds and addition of 1.5% propionic acid, followed bythe treatment of addition of 1.5% propionic acid, then the unirradiated treatment and the least degradation in aflatoxin concentration occurred in the sesame halva processed from the irradiated contamined sesame seeds.

Table 1. Frequency of the occurrence of different fungal species isolated from raw sesame seeds and their ability to produce ***aflatoxins***.

Isolated fungal species	Freq. (%)	*Aflatoxins mg/L*			
		B1	B2	G1	G2
Aspergillus flavis (Br)	1515	410	270	225	170
Aspergillus flavis (Lg)	20	70	50	130	-
Aspergillus niger	25	-	-	-	-
Penicillium spp. (Bg)	40	-	-	-	-

(Br): Brown colour (Lg): Light green colour. (Bg): Blue green colour.

Table 2: Aflatoxin concentrations in different contaminated sesame seed treatments and processed sesame halva.

Samples	Alfatoxin concentrations (mg/Kg)							
	B1	Red.%	B2	Red.%	G1	Red.%	G2	Red%
a) Contaminated sesame seeds*								
Unirradiated sesame seeds (control) (s1)	21500	-	3750	-	32500	-	800	-
Irradiated sesame seeds (10 Kgy) (s2)	25000	+16.3	4000	+6.7	35000	+7.7	1250	+5 6.3
Unirradiated seeds 1.5% propionic a. (s3)	13500	37.2	380	89.9	2500	92.3	350	56.3
Irradiated seeds+ 1.5% propioic a. (s4)	20000	20.0	3600	10.0	27000	22.9	960	23.2
b) Sesame halva processed from contaminated sesame seeds								
Unirradiated sesame seeds (control) (sh1)	18730	12.9	3500	6.7	30500	6.2	300	62.5
Irradiated sesame seeds (10 Kgy) (sh2)	23500	6.0	3850	3.75	33200	5.1	950	24.0
Unirradiated seeds 1.5% propionic a. (sh3)	11700	13.3	250	34.2	2320	7.2	160	54.3
Irradiated seeds+ 1.5% propioic a. (sh4)	2000	90.0	3300	8.3	21000	22.2	500	47.9

* Sesame seeds were inocilated with the mixed fungal culture of ***Aspergillus parasiticus*** NRRL 2999 A***spergillus flavus*** EMCC 274 after being treated with the different treatments.

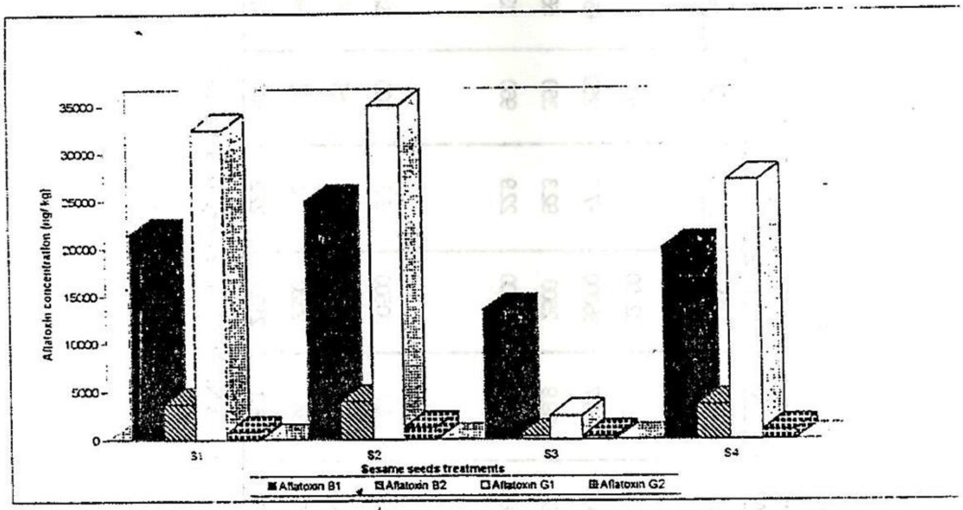


Fig. 1. Aflatoxin production by the mixed fungal culture of *Aspergillus parasiticus* NRRL 2999 and *Aspergillus flavus* EMCC 274 in sesame seeds of different treatments.

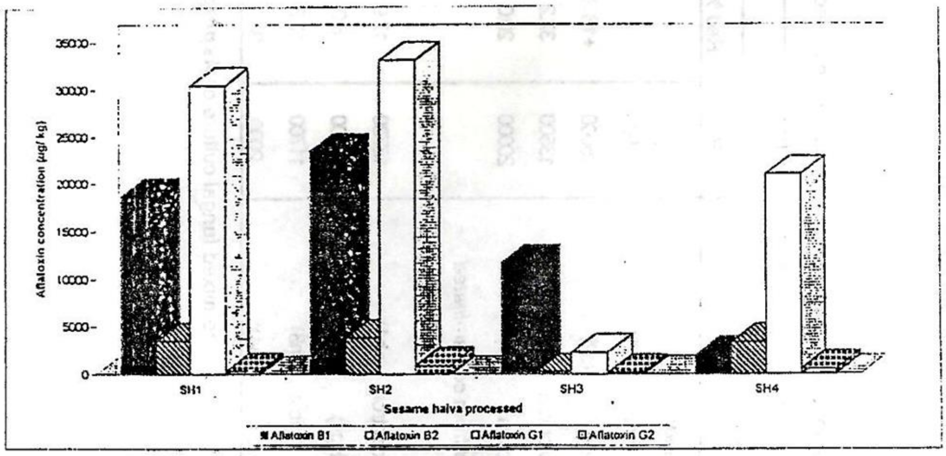


Fig. 2. Aflatoxin concentrations (mg/kg) in sesame halva processed from different treatments of sesame seeds noculated with the mixed fungal culture of *Aspergillus parasiticus* NRRL 2999 and *Aspergillus flavus* EMCC 274.

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الأفلاتوكسينات في بذور السمسم الملقحة والحلاوة الطحينية المصنعة منها

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تمت دراسة وجود ونمو الفطريات المفرزة للأفلاتوكسينات في بذور السمسم وإمكانية التحكم في الأفلاتوكسين الناتج في الحلاوة المصنعة منها.

أظهرت النتائج وجود فطر *Penicillium* بنسبة مرتفعة (٤٠٪) في البذور الخام للسمسم ثم فطر *A.niger* بنسبة ٢٥٪ وكانت هناك سلالتين من فطر *A.flavus* بنسبة ٣٥٪ وكانت منتجة للأفلاتوكسينات.

تم معاملة بذور السمسم بأحد المعاملات التالية للتحكم في النمو الفطري وإنتاج الأفلاتوكسين وهي:

١– التشعيع بطاقة 10 KGy.

٢– إضافة ١,٥٪ حامض البروبيونيك.

٣– التشعيع وإضافة حامض البروبيونيك معاً، وتم مقارنة بذور السمسم المعاملة مع العينة الكنترول (الغير معاملة) لتقدير الأفلاتوكسينات الناتجة من تلقيح بذور السمسم بكل من جراثيم فطر *A. parasiticus* و*A. flavus* النقية.

ولقد لوحظ أن اشعاع بذور السمسم بمعدل 10 KGy قبل عمليات التلقيح بالفطريات النقية ادى إلى زيادة الأفلاتوكسينات المنتجة، وقد يرجع ذلك إلى التأثير الأشعاعي على التخلص من جميع الكائنات الدقيقة المهاجمة وبالتالي وفرت فرصة النمو والنشاط للفطريات الملقحة النقية.

ولكن باضاة ١,٥٪ حامض البروبيونيك للبذور المشعة قد انخفض انتاج الافلاتوكسينات بها.

وباستخدام بذور السمسم الملوثة بسلالتي فطر *Aspergillus* في تصنيع الحلاوة الطحينية ظهر انخفاض واضح في نسبة الافلاتوكسينات المتكونة. وكانت أعلى نسبة في الانخفاض ٩٠٪ للأفلاتوكسين B_1 عند إنتاج حلاوة طحينية من بذور سمسم مشععة ومضاف لها ١,٥٪ حامض البروبيونيك.