

Suitability of Some Foodstuffs for Aflatoxin Production

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ABSTRACT. Ten different foodstuffs consumed in several countries of the Middle East were tested for their suitability as substrates for aflatoxin production. Out of these, pine nut and Licorice Root (Sweet Root = *Glycyrrhiza glabra*) were found to favour the production of aflatoxin, which make them, under certain conditions, a source of risk.

Aflatoxins, a group of toxic metabolites produced by certain *Aspergillus* species, have been found to be carcinogenic, teratogenic and mutagenic to several species of experimental animals (Butler and Barnes 1968, Goplan *et al.* 1972, Adamson *et al.* 1973). Aflatoxin B₁ is the most potent hepatocarcinogenic so far recognized (Wogan and Newberne 1967) and is suspected of being a primary cause of human cancer in some population groups (Newberne and Rogers 1973). Aflatoxins occur in a variety of crops, meat, milk and eggs of animals that consumed aflatoxin containing feed.

Material and Methods

Ten foodstuffs (Table 1), were tested for aflatoxin production. Spores of *Aspergillus parasiticus* NRRL 2999 were inoculated in a tube of potato-dextrose agar and incubated at 28°C for 7-10 days, until sporulation. Flasks containing 50 g of the foodstuff and 25 ml of distilled water were autoclaved and seeded with 2 ml of spore suspension containing 10⁵-10⁷ spores/ml. Flasks were placed in an orbital incubator, set at 28°C and 185 rpm. Aflatoxins were extracted by adding chloroform to the cultures at the end of the incubation period, blended for 5 min and centrifuged for 15 min at 3,000 rpm. The chloroform layer was passed through

anhydrous sodium sulfate and then evaporated to dryness. The residue was then dissolved in 1-2 ml of chloroform. Aflatoxins were identified on the basis of Co-migration with standards of aflatoxin B₁ and B₂ and their fluorescent color, under long UV illumination. The fluorescent aflatoxin bands were scraped off and extracted from the silica gel by methanol for the UV absorption, using Hitachi 200 spectrophotometer, and compared to the UV absorption of aflatoxin standards. Aflatoxin concentrations were calculated according to Masri *et al.* (1968) method. Moreover, confirmatory tests were made according to the method by Stoloff (1967). Rice and Peanut, well known substrates for aflatoxin production (Shotwell *et al.* 1966, Conduer *et al.* 1963) were used as reference.

Results

As indicated in Table 1, the suitability of the tested foodstuffs for aflatoxin production varied greatly.

Pine nut and Liquorice Root proved to be good substrates for fungal growth and aflatoxin production. Though dried okra, anise, khoudary flowers and Marian plant were found to be good substrates for the fungal growth, no aflatoxin was produced in them. Malta jute, palm date and safron gave little fungal growth, even after incubation for 10 days. Moreover, the fungus sporulated in most of the tested foodstuffs.

Pine nut allowed maximum yield of aflatoxins B₁ and B₂ per a gram of seed. Amount of aflatoxins formed in this substrate was 71 μ g and 11 μ g of aflatoxins

Table 1. Suitability of some foodstuffs for *Aspergillus parasiticus* NRRL 2999 growth and aflatoxin production.

Substrate	Fungal growth	Aflatoxin production
Pine nut	+++	+++
Liquorice Root	+++	+
Okra	++, S	-
Malta jute	+, S	-
Palm date	+, S	-
Safron	+, S	-
Anise	+++	-
Marian Plant	++, S	-
Khoudary flowers	++, S	-
Kamerdeen	-	-

+++ = high, ++ = moderate, + = scant and S = sporulation

Table 2. Amount of aflatoxins produced by *Aspergillus parasiticus* NRRL 2999 on various substrates.

Substrate	Aflatoxin production ($\mu\text{g/g}$ substrate)			
	B ₁	B ₂	G ₁	G ₂
Peanut (positive control)	76	13	6	Traces
Rice (positive control)	100	7	11	Traces
Pine nut	71	11	5	Traces
Liquorice Root	0.95	–	–	–

B₁ and B₂, respectively. Such amounts of aflatoxin yields, in comparison with that of the control substrates (peanut and rice) are high (Table 2). On the other hand, aflatoxin production in Liquorice Root was at the rate of 0.95 μg per gram substrate of aflatoxin B₁ only.

Discussion

Out of the ten different foodstuffs which are widely consumed in several countries of the Middle East, Pine nut and Liquorice Root (*Glycyrrhiza glabra*) were found to have a good potential for aflatoxin production. Pine nut is used in many traditional foods, and thus is often stored in houses. This study has definitely indicated pine nut to be a good substrate for aflatoxin production. When grown on pine nut, the aflatoxigenic fungus produced high amounts of aflatoxins B₁ and B₂. Pine nut, rice, and peanut substrates yielded even a higher amount of aflatoxin B₁. Rice and peanut are known to be good substrates for aflatoxin production (Shotwell *et al.* 1966, Conduer *et al.* 1963).

Liquorice Root (*Glycyrrhiza glabra*) is commonly used in most of the Arabic countries, for the preparation of a soft drink. Thus, the ability, of the aflatoxigenic fungi, to produce a rather low level of aflatoxin B₁ on it is fairly assuring.

Other foodstuffs were found to be unfavorable for aflatoxin production. The unsuitability of these substrates for aflatoxin production may be due to one or more of several factors, *i.e.* carbohydrate and mineral content, or presence of inhibitory agents, *viz.* phytic acid or hydroxycinnamic acid and others.

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ملاءمة بعض المواد الغذائية لإنتاج الأفلاتوكسين

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لقد تم في هذه الدراسة فحص عشر مواد غذائية تستهلك
بكثرة في معظم أقطار الشرق الأوسط، حول مدى ملاءمتها
لإنتاج سموم الأفلاتوكسين.

أوضحت هذه الدراسة أن الصنوبر وسط ملائم جدا
لإنتاج سموم الأفلاتوكسين ب₁ و ب₂، وباقي المواد غير
ملائمة لإنتاج هذه السموم، بالرغم من أن الأفلاتوكسين
ب₁ قد تكوّن في عرق السوس بكمية قليلة جدا.