Aflatoxin Production in Peanut Varieties by *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare

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Levels of aflatoxin produced in peanuts differed with the genetic variety of plant and with the species and strain of invading fungus. Possibilities for identifying groundnut varieties partially resistant to aflatoxin production are discussed.

Aflatoxin contamination in food grains is now well recognized as a public health hazard (6). Several approaches towards alleviation of the problem are being attempted (6). One promising method is to obtain varieties of peanuts (*Arachis hypogaea* L.) (12, 13; A. Z. Joffe, Final Rep. PL. 480 Project, 1968, p. 142, the Hebrew Univ. of Jerusalem, Israel), sorghum (R. J. Anandam, M.Sc. Ag. thesis, A.P.A.U., Hyderabad), and corn (10) that do not serve as favorable substrates for the production of aflatoxins. In the first report of genetic resistance of peanuts to aflatoxin production, Rao and Tulpule (12) screened 60 varieties of peanuts; no toxin was produced on one variety, US-26 (PI. 246388), although growth of the fungus in the kernels was normal. On the other hand, Howell (8) and Doupnik (4) reported that aflatoxin was produced in variety US-26. Doupnik's (4, 5) data, however, indicate wide variations in toxin production among the 20 breeding lines of peanuts investigated.

In view of the importance of the genetic approach for the prevention of aflatoxin accumulation in stored peanuts, an attempt was made to study in greater detail the interactions of plant variety and species and strain of fungus on aflatoxin production.

Aflatoxin production was examined in two varieties of peanut, TMV-2 (the most common Indian variety) and US-26 (PI. 246388). Three isolates of *Aspergillus flavus*, (NIN 25, NIN 163, NIN 169) and two isolates of *Aspergillus parasiticus* (NRRL 2999 and RIB 4002; designated as *Aspergillus toxicarius* by Murakami [9]) were used. The toxin-producing potentials of these isolates were first graded on a synthetic medium described by Adye and Mateles (1). Twenty-four gram lots of each variety of peanut were rehydrated with 10 ml of water, sterilized at 121 C for 15 min, and inoculated with 1 ml of a spore suspension (approximately $6 \times 10^4$ spores/ml) of a fungus. The flasks were incubated at 28 C for 7 days, sprayed with 95% alcohol, and dried overnight at 80 C. The dried samples were first defatted with n-hexane and then extracted with methanol. The aqueous methanol extracts were treated with basic lead acetate for removal of pigments. The toxins were then extracted into chloroform and the CHCl₃ extracts were appropriately processed for screening by thin-layer chromatography using CHCl₃-MeOH (95:5) as the developing solvent system. Aflatoxin B₁ was quantitated as described by Pons et al. (11). This method is capable of detecting as little as 0.3 part per billion (ppb) of aflatoxin B₁. Chemical confirmation of aflatoxin was made by spraying the chromatograms with 10% HCl in ethanol, as described by Crisan (2).

Toxin production by different fungal isolates varied considerably as is shown in Table 1. Isolates of *A. flavus* used in this study produced only B₁ and B₂, whereas *A. parasiticus* produced B₁, B₂, G₁, and G₂. The two species can be readily determined by light microscopy. The conidia of *A. flavus* show only minute echinulation as compared to prominently echinulate conidia of *A. parasiticus*.

The results indicate clearly that there are species and varietal differences in toxin production (Table 2). The variation in toxin production appears to be intimately related to the inherent ability of the fungal isolate to produce the toxin. The fungal isolate used by Rao and Tulpule (12) was perhaps so low in toxin-pro-
Table 1. Aflatoxin production of the fungal isolates used on synthetic medium

| Isolate       | Conidial character | Quantitative | Qualitative |
|---------------|--------------------|--------------|-------------|
| NIN-25 A. flavus | Minutely echinulate | +*           | B₁, B₂      |
| NIN-163 A. flavus | Minutely echinulate | ++           | B₁, B₂      |
| NIN-169 A. flavus | Minutely echinulate | +++          | B₂          |
| NRRL-2999 A. parasiticus | Prominently echinulate | ++++++       | B₁, B₂, G₁, G₂ |
| RIB-4002 A. parasiticus | Prominently echinulate | ++++++       | B₁, B₂, G₁, G₂ |

* +, Equivalent to 250 μg of B₁/100 ml of medium.

Table 2. Toxin produced in the two genotypes of peanuts (TMV and US-26) by five Aspergillus spp.

| Isolate       | B₁ in picograms per gram |
|---------------|--------------------------|
|               | TMV      | US-26   |
| NIN-25 A. flavus | 250           | 25       |
| NIN-163 A. flavus | 1,500        | 1,000    |
| NIN-169 A. flavus | 2,120        | 1,000    |
| NRRL-2999 A. parasiticus | 51,600     | 25,000   |
| RIB-4002 A. parasiticus | 80,000     | 25,000   |

Producing capability that no detectable toxin could be detected when it was grown on US-26, although measurable amounts of the toxin were produced on other varieties of peanuts. The toxin production was also related to the species of Aspergillus used; A. parasiticus always produced greater amounts as compared to A. flavus. Such differences have also been observed on liquid media and other natural substrates (7). Despite these variables, the difference in toxin production attributable to the genotype was always demonstrable. This is also apparent from the data of Doupnik and associates (4, 5).

It is intriguing that certain varieties of peanuts support low toxin production, whereas other varieties support maximal production. This difference is possibly related to certain basic biochemical characters such as protein (10) or possibly vitamin E (3) besides cultivar practices.

From the point of view of prevention, the challenge, therefore, appears to be to identify peanut genotypes that will support minimal toxin production by a number of fungal isolates of A. flavus and A. parasiticus. A. parasiticus is well recognized to be powerfully toxigenic. The prevalence of A. parasiticus in stored food grains appears to be not as sufficiently well investigated as A. flavus, although Hesseltine et al. (7) suggest that the occurrence of A. parasiticus is restricted primarily to the tropics. However, in our preliminary studies on more than a hundred isolates of Aspergilli from stored peanuts and other food grains, A. parasiticus was not encountered. An exhaustive study by Raghaven德拉 Rao et al. (Final Report US-PL. 480 Project, Regional Research Labs, Hyderabad, India, 1970) on cotton seed mycoflora, involving about 2,500 isolates, also did not reveal the presence of A. parasiticus. The rare occurrence of A. parasiticus contamination of grains in India, as revealed by these limited studies, is indeed encouraging because aflatoxin accumulation on stored food grains should be considerably less than if A. parasiticus was the predominant species.

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