# Technical News Feature



# Unavoidable Low Level Aflatoxin Contamination of Peanuts

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## **ABSTRACT**

A major portion of aflatoxin contamination of peanuts probably occurs when decayed or discolored peanuts are incompletely removed by sorting. Quality control measures have been instituted in the United States to insure that unavoidable aflatoxins in consumer peanuts and peanut products do not exceed 20 μg/kg. However, low level aflatoxin contamination, from trace amounts to about 50  $\mu$ g/kg in sound mature unblemished peanuts, can occur before peanuts are dug. This low level contamination is not related to high levels of Aspergillus flavus infection or to current production practices. Low level aflatoxin contamination of peanuts may be endemic, and current sorting procedures may not be effective in removing unblemished contaminated peanuts.

#### INTRODUCTION

Aflatoxin contamination of peanuts has been of worldwide concern since the 1960s. The aflatoxins are secondary metabolites of Aspergillus flavus Link ex. Fr. and Aspergillus parasiticus Speare. Research has shown that these fungi can infect peanuts and produce aflatoxins before digging, in the windrow, after harvest, and in storage (1).

Several factors are assumed to be important in favoring aflatoxin contamination of peanuts before they are dug. These include insect and mite damage, drought stress, irrigation practice, maturity stage, mechanical damage, climate during harvest, and growth cracks (1). For example, irrigation plots to 50  $\mu$ g/kg in irrigated plots in Texas (2) and from 128  $\mu$ g/kg to undetectable in India (3). Dickens et al. (4) speculated from North Carolina observations in 1968 that injury by lesser cornstalk borer and drought increased visible A. flavus contamination of peanuts. Aucamp (5) demonstrated that mites could be vectors of A. flavus, and Porter and Smith (6) found that total fungal colonization of peanut pods was enhanced by injury from southern corn rootworm larvae, but that A. flavus colonization was not affected. Griffin (7) showed that A. flavus spores do not germinate well around uninjured peanut pods, but germination was enhanced when the pods were injured. Over-mature pods and those with broken shells or pods with growth cracks were more likely to contain aflatoxins than sound mature kernels (8).

Much of the work on aflatoxin contamination of peanuts has been with conditions favoring high levels of aflatoxin contamination concentrated in relatively few damaged peanuts and ways to prevent contamination. However, in some cases aflatoxins from trace amounts to about 50  $\mu g/kg$  are found in commercial lots of peanuts that are difficult to reprocess to lower the aflatoxin levels (9). The purpose of this investigation was to determine if there is a detectable background level of aflatoxins in sound mature peanuts at the time the peanuts are dug.

# **EXPERIMENTAL PROCEDURES**

The peanuts were grown at Tifton, Georgia, using normal production and harvesting practices. All peanuts were Segregation I grade peanuts. Peanuts were harvested from randomized split-plot rotation plots replicated four times that were either in continuous peanut, or 2, 3, or 4 year rotations with corn, soybeans, or cotton (peanut-corn-cornpeanut). Rye was used on half the main plot as a cover crop whether it be continuous peanuts or 2, 3, or 4 year rotations. Samples were also taken from four replications of randomized variety trials of commercially grown cultivars. and others were from four replications of randomized check plots that had received recommended fertilizer rates in nutritional studies.

Samples were collected from each of the four replications in 1973, 1974, 1975, and 1976. Each year 5-lb samples of sound peanuts from each plot were shelled, hand sorted to remove damaged and immature kernels, ground, and 50-g subsamples were analyzed for aflatoxins using the AOAC Method I (10). In 1973 and 1974, infections of sound mature seed and surface contamination of pods were assayed by plating either whole seed, or a 50-pod sample from a plot was rinsed with 1000 ml of water, and 0.5 ml of the rinse water was placed onto M3S1B agar medium (11); in 1975 and 1976, one replication from each treatment was checked for infection by the A. flavus group. The relative aflatoxin production of 43 isolates recovered from seed was determined by incubating the isolates for 7 days on autoclaved 25% moisture peanuts.

TABLE I Incidence of Aflatoxins in Peanut Samples From Production Plots

| Year | Number of plots <sup>a</sup> | Number positive <sup>b</sup> | Range <sup>c</sup> | Average level of positives <sup>d</sup> | Average of all plotsd |
|------|------------------------------|------------------------------|--------------------|---|-----------------------|
| 1973 | 38                           | 21                           | tr-15              | 7                                       | 4                     |
| 1974 | 107                          | 33                           | tr-60              | 13                                      | 4                     |
| 1975 | 90                           | 20                           | 6-182              | 30                                      | 7                     |
| 1976 | 110                          | 0                            |                    | 0                                       | Ö                     |

a 50 g Subsamples were tested from each plot.

bNumber of plots with aflatoxins.

<sup>c</sup>Range of aflatoxins found  $B_1 + B_2 + G_1 + G_2$  ( $\mu g/kg$ ).

dAverage of aflatoxins content,  $B_1 + B_2 + G_1 + G_2$  ( $\mu g/kg$ ).

### **RESULTS AND DISCUSSION**

The incidence of aflatoxin in seed from plots, seed infection by the A. flavus group, and A. flavus propagules on pods occured at random and was not statistically related to any variety or production practice. Therefore, all data were pooled. The variety trials and nutritional studies indicated that commonly grown cultivars were contaminated by low levels of aflatoxins in a random manner (Table I).

There was a low level of seed infection by the A. flavus group when the peanuts were dug. In 1973 0.9% of 8000 seed contained A. flavus, in 1974 1.4% of 3000 seed contained A. flavus and, in both 1975 and 1976 0.6% of 2000 seed contained A. flavus when seed were surface-sterilized for 2 min with 0.5% aqueous sodium hypochlorite and incubated on M3S1B medium for 5 days at 30 C. Both 'Florunner' and 'Florigiant' had similar low levels of infection. The A. flavus group recovery was random and not statistically related to any variety or production practice. In 1973, A. flavus group isolates from infected peanuts were tested for aflatoxin production; 21 isolates produced more than 1000  $\mu$ g/kg. Twenty-one isolates produced between 50 and 1000  $\mu$ g/kg, and one isolate produced traces of aflatoxins when incubated for 7 days on autoclaved peanuts adjusted to 25% moisture.

The average number of propagules of the A. flavus group isolates from pod rinses was not significantly different for Florunner and Florigiant and production practice in either 1973 or 1974. About 100 colonies per pod were recovered in 1973 and 70 colonies per pod in 1974 when 50 pods per plot were rinsed in 1000 ml H<sub>2</sub>O, and 0.5 ml of rinse water was plated on M3S1B medium. In 1975 and 1976, 20 randomly selected plots were assayed in this manner, and 70 and 90 colonies were found in these plots respectively.

These experiments were designed to survey the unavoidable aflatoxin contamination in peanuts grown under normal production practices. The data indicate that there is contamination that cannot be related to production practices, varieties, or visible damage. There are differences from year to year that cannot easily be explained. It would be helpful to know why the 1976 plots had no aflatoxin contamination, and in 1973, 1974, and 1975 some plots had some aflatoxin contamination in a random manner, There was sufficient A. flavus inoculum on the pods in all plots tested to insure infection of peanuts under favorable conditions for A. flavus growth, but there was never over 1.5% infection of the seed at harvest. With this level of seed infection, it is difficult to relate A. flavus infection with aflatoxin contamination. These data revealed that A. flavus infection and aflatoxin contamination both occurred independently at random.

The literature on experiments comparing such factors as drought stress, effects of growth cracks, and effects of insect damage clearly shows that high levels of aflatoxins can be encountered, but many times they can be avoided by management. The practical implication of low level unavoidable contamination is that we probably cannot totally eliminate aflatoxin contamination of sound unblemished

peanuts. Our results indicate that there is a background level of aflatoxins in sound peanuts with low levels of A. flavus infection in the field. Dickens and Whitaker (9) demonstrated that repeated electronic sorting and hand picking would remove 50 to 75% of the aflatoxin in lots containing about 48  $\mu$ g/kg, but the efficacy with electronic sorting was highly variable. The data on aflatoxin contamination, from trace amounts to 50  $\mu$ g/kg, of sound peanuts presented by Jackson (12), Joffe (13), Doupnik (14), Pettit et al. (2) Subrahmanyam and Rao (3), McDonald and Harkness (15), and Beuchat et al. (16) support this view. Therefore, the major part of the aflatoxins found in surveys such as the Food and Drug Administration surveys of peanut products (17) and the Peanut Administration Committee Testing Program (18) may be endemic in sound peanuts and may be currently unavoidable with present practices. The emphasis on removing damaged pods and seeds in the peanut industry has done much to minimize aflatoxin contamination. However, there is a continuing problem of low level aflatoxin contamination that is not necessarily associated with damage or discoloration. Appropriate allowances for this type of aflatoxin contamination should be made in research objectives as well as in commercial practice to determine the causes of aflatoxin contamination of apparently sound peanuts.

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