THE PRESENCE OF AFLATOXIN IN KERNELS FROM FIVE YEARS GROUNDNUT CROPS AND OF *ASPERGILLUS FLAVUS* ISOLATES FROM KERNELS AND SOILS *

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SUMMARY

In T.L.C. tests for 605 samples of groundnut kernels from 5 years' yield, the percentage of fresh kernels in which aflatoxin was present was very low (up to 6.4%), while that of stored kernels ranged from 0 to 32.0%. But the intensity of toxicity was invariably very low (up to 125 ppb).

Of 1626 *Aspergillus flavus* isolates from groundnut kernels rhizosphere and geocarposphere, and from soil in which groundnuts grew, about 90% were found capable of forming aflatoxin. In quantitative tests with 750 isolates 60% of the isolates produce aflatoxin in excess of 25,000 ppb.

In 5 years' work on groundnuts, a study has been made of the toxicity of fresh and stored kernels, and of the *Aspergillus flavus* isolates made from such kernels and from the soil in which the groundnut crops were sown. In 1963 only stored kernels were tested, in 1964–1967 both fresh and stored kernels.

METHODS

Soil samples were taken from fields in which groundnuts had been sown. Each sample consisted of soil collected in a sterile manner from 8 spots in each field. Each sample was weighed and diluted with 0.75% NaCl solution to give final dilutions of 1:500 and 1:5000. Five petri dishes with acidified Czapek's medium and 5 dishes with Sabouraud's medium were inoculated with 0.5 ml from each dilution. The plates were incubated at 24°C.

In 1966 and 1967 samples were also taken from the geocarposphere and rhizosphere of groundnuts.

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After sterile withdrawal of the roots and geocarps (pods) from the flasks, the weight of the soil left in the flasks was determined. It was diluted to 1:2000 and introduced into petri-dishes with Czapek's medium and Sabouraud's medium.

Where isolates of *A. flavus* developed in any of the above samples, they were transferred to slants.

Samples of fresh and stored kernels were examined as follows: Geocarps and kernels were disinfected with 0.1% mercuric chloride. One hundred kernels were sown in 25 petri dishes with Czapek's medium.

Isolates of *A. flavus* from soils and kernels were sown on a wheat medium (10 g wheat, 10 ml tapwater and traces of ZnSO₄). After 8 days incubation at 24°C the fungus was killed with chloroform.

Aflatoxin extraction and determination in kernels from the 1963, 1964, and 1965 yields and on soil samples taken in these years, were carried out by the method described by Campbell *et al.* 1, Trager *et al.* 11 and Nesheim 7. This method serves mostly for qualitative indication of the presence or absence of the toxin.

From the kernels harvested in 1966 and 1967 aflatoxin was extracted and quantitatively determined by the more sensitive method described by Pons *et al.* 8. The method was slightly modified by (a) substituting a 85:15 chloroform:acetone mixture for the 97:3 chloroform:methanol recommended originally for use as developing solvent, and (b) not lining the development tank with filter paper.

**RESULTS**

*Frequency and intensity of toxicity in groundnut kernels*

Toxicity tests were carried out on 186 samples of fresh kernels, not more than 24 hours after they had been harvested, and on 419 samples of kernels stored for 2-8 months. Each sample consisted of 50 grammes of kernels.

Table 1 indicates the frequency of toxicity in these samples in 1964-1967: – In fresh kernels, only 0-6.4 per cent of the samples were found toxic, while in stored kernels the frequency varied more markedly, from 0 and 0.9 in 1965 and 1966 to 12.7 per cent in 1964 and 32 per cent in 1967.

The intensity of toxicity in fresh samples was always low and did not exceed 57 ppb.

Accurate determinations of the intensity of toxicity were made on the stored kernels of the 1967 yield. Results are presented in Table 2. They show that only 3.3 per cent of the samples were in the toxicity range of 60-125 ppb, which in itself is quite low, while all other samples were either non-toxic or very weakly toxic.