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ELABORATION OF AFLATOXINS ON RAW GROUNDNUTS IN PAKISTAN

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Analytical studies of the raw groundnuts from various places were carried out to determine the incidence of aflatoxins. Aflatoxin assays on fifty raw groundnut samples showed that three samples were contaminated. Samples below 16% moisture showed absence of alfatoxins. Isolation from samples showed that 37.5% isolates of *Aspergillus flavus* were toxigenic and capable of producing aflatoxin *in vitro*. None of the sample was resistant to the production of aflatoxin when growth of the toxigenic fungi was experimentally induced.

INTRODUCTION

In Pakistan groundnut crop is grown over an area of 41,000 hectares (1974-75); Pindi Gheb, Chakwal, Gujjar Khan and Fateh Jhang areas being the principal zones of cultivation and production. Almost all the nuts are consumed as dry fruit after roasting. This may pose a health hazard if the groundnuts are contaminated. Hence this investigation was undertaken to determine the incidence of aflatoxins in the raw groundnuts. The relationship of high moisture with aflatoxin occurrence and the toxigenic fungi present on the samples was also investigated. The fungal isolates were further tested for their toxigenic character.

MATERIALS AND METHODS

Collection of Groundnuts. Fifty of groundnuts samples were collected between the months of November to February from the grain markets of Lahore, Faisalabad and Sahiwal in cloth bags for analytical studies. Each sample weighed between 3-4 kg.

The groundnuts were shelled manually and the kernels were crushed in a Moulineux coffee grinder Type MCOC. The meal was partially defatted with hexane in a soxhlet. It was thoroughly homogenised and was further ground and passed through 14 mesh sieve (British Standard 410:1943). The sample was quartered and a 50 g representative sample taken up for analytical studies.

Moisture. The moisture content of all the samples was determined in an oven at 105° until constant dry weight was obtained.

Preparation of Inoculum. It was prepared by inoculatig tubes of potato-dextrose-agar[1] with spores of a locally isolated toxigenic strain of *Aspergillus flavus.*

Experimental Contamination. One ml of the above spore suspension was grown in aseptic conditions on 40 g of healthy kernels for 10 days. The fungus was destroyed by brief steaming and the material was tested for the presence of aflatoxins.

Isolation of Mycoflora. The kernels were removed aseptically, placed on potato-dextrose-agar and incubated at 30 ± 2° temperature and examined daily for the presence of fungi for ten days. Representative colonies were picked up and purified by subsequent subculturing.

Taxonomical Studies of the Mold. Taxonomical studies of the pure cultures obtained by the above procedure were based on cultural characteristics as well as microscopic examination. The results obtained were compared with those of Thom and Rapers[2].

Screening for Aflatoxin Production. The isolates ability to produce aflatoxin B_1 *in vitro* was studied using the method of Davis *et al.*[3].

Detection of Aflatoxin. The Romers minicolumn method[4] was employed for the detection of aflatoxin.

Confirmation of Aflatoxin. Positive samples were confirmed on TLC plates using the Przyblskis procedure[5].

RESULTS AND DISCUSSIONS

The moisture content of samples collected from the Grain Market Lahore varied from 10.5 to 17.0%. Only two contaminated had a moisture content above 16 per cent wheras the samples from Faisalabad have a moisture range of 10.5 to 14.9 per cent. Out of the 15 samples from Faisalabad only one had moisture above 14.0 per cent. There was no contamination in the samples from Faisalabad. The variation of moisture from Sahiwal samples were from 11.5 to 16.0 per cent. Only one sample, having moisture content above 16 per cent was found to be contaminated with aflatoxin.

The presence of aflatoxins in moldy groundnuts and groundnut meals has been demonstrated by Allcroft and

Table 1. Number of infected samples.

Isolates	Lahore	Faisalabad	Sahiwal
Aspergillus flavus group	7	5	4
Aspergillus Niger group	10	8	
Mucor Sp.	2	6	8
Fusarium Sp.	5	4	6
Alternaria Sp.	1	2	4
Penicillium Sp.	8	7	12
Monilia Sp.	1	1	2
Pullularia Sp.	2	3	4
Beltrania	–	2	1

Carnaghan[6] and Tulpule *et al.*[7]. *Aspergillus flavus* infests the groundnut crop under conditions unfavourable for collection, storage and transport of the crop. Pattinson *et al.*[8] observed that under tropical conditions, the mold can grow rapidly on groundnuts which have been dried slowly following lifting, or on nuts which have been shelled immediately following lifting or on kernels which have been damaged by disease during growth or mechanically damaged during harvesting operations[9-11]. Higher moisture level in groundnuts provides favourable environment for the fungal growth and production of aflatoxin. According to Harrison[12], absolute safety from even slow mold growth could be achieved at about 14.0 per cent moisture and that progressively higher moisture allowed increasing number of species of mold can be seen. Present findings confirm the observations of Harrison[12].

Fungal flora present on the raw groundnut samples are given in Table 1. Out of the seven isolates of *Aspergillus flavours* from Lahore samples three were found capable of aflatoxin production; out of five isolates of *Aspergillus flavus* group from samples collected from Faisalabad only one toxigenic; similarly out of four isolates of *Aspergillus flavus* group from Sahiwal two were found to be toxigenic in character when tested *in vitro*. The results Table 1) presented herein suggest that the Fungi are quite prevalent on the groundnuts in Pakistan and that out of a total of sixteen isolates of *Aspergillus flavus* group, six were capable of producing aflatoxin *in vitro*. This indicates the possibility of aflatoxin production on groundnuts under favourable moisture and temperature conditions.

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