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A Study of Groundnuts from the Western Area of Libya

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INTRODUCTION.

The groundnut, Arachis hypogaeaL., grows well in Libya and the nuts are usually used for human consumption. There does not seem to be any reason why production should not be increased to produce edible oil. The "meal" left after oil extraction is a valuable feeding stuff for animals, in particular poultry.

Unfortunately. groundnuts are an excellent growing medium for the common stored product mould A.flavus in particular when they are badly harvested or improperly stored. A.flavus, under suitable conditions produces a group of structurally similar and very toxic metabolites, the 'aflatoxins'. These compounds are the most potent known carcinogens and are particuarly toxic to birds.

Analysis for aflatoxins is carried out by bioassay using one day old ducklings or by chromotographic techniques. For speed and accuracy, T.L.C. methods are now favoured, however for the work described below; suitable apparatus was not at the time available. paper chromatographic methods were therefore used.

MATERIALS AND METHODS.

Groundnut samples, unshelled, were supplied by the Agricultural Bank, Tripoli from the 1970 harvest. The samples, ca250g, were immediately examined for signs of damage to the shell. From ten of the original samples, ground nuts (ca 25g) were extracted mechanically from the shells and coarsely ground in a "Waring" blender.

Determination of oil content and preparation of chloroform extracts.

The samples,described above, were extracted to constant weight with light petroleum, b.p60-80°. The meal was then air dried and extracted with chloroform for six hours. The chloroform extracts were evaporated to dryness and the residue dissolved in chloroform (ca 1 ml).

Examination of chloroform extracts by paper chromatography.

Ascending chromatography using "Schleicer and Schull" 2040b paper was carriod out. The solvent systems were :

Solvent System A. n-butanol/glacial acetic acid,95 to 5.

Solvent System B. Benzene-toluene-cyclohexane-ethanol-water (3:3:5:8:5 by volume). Upper layer, with glacial acetic acid added (1 volume added to every 100 volumes).

Fluoroscent spots were detected using long wavo ultra-violet absorbtion.

Preparation of a standard solution of Aflatoxin Bl.

A standard meal, containing ca 8ppm Aflatoxin Bl was extracted with chloroform for six hours. The chloroform solution was evaporated to dryness and the residue dissolved in chloroform (5ml).

SAMPLE No.	SOURCE	VARIETY	EVIDENCE OF DAMAGE or Fungal Attack.	OIL CONTENT	SOLVENT SYSTEM A Fluorescence	RfX10	SOLVENT SYSTEM B Fluorescence	RfX10
1	Zanzur	Vir.	+ + +	42.5 %	—	—	—	—
2	Abu-Essa	Vir.	+	48.5 %	Blue (W)	5.7	Blue	6.1
3	Zavia	Val.	+ + +	44.5 %	Blue (S)	7.0		
4	Swani-ben-Yaden	Vir.	+ +	44.5 %	Blue (VW)	7.0		
5	El-arawi-Zavia	Vir.	+ + +	47.0 %	Blue (VW)	7.0		
6	El-Maih	Val.	+ +	47.5 %	—	—		
7	El-Ameria	Vir.	+	50.5 %	—	—		
8	El-Nasoria	Vir.	+	50.0 %	Blue (S)	6.8	Blue	6.7
9	El-Gerian (School)	Val.	+ + +	44.0 %	Blue (W)	6.8	Blue	6.9
10	El-Agalat	Vir.	+	52.0 %	Blue (S)	7.6	Blue	5.0
Control	——	——	——	——	Blue	6.8	Blue	7.0

a). Variety : Vir = Virginia, Val = Valencia.

b). Damage: + + 10% , + 2-10%

c). Aflatoxin BI Rf All corrected.

d). S = strong, W = weak, VW = very weak.

e). With solvent System B all other Rf values were corrected.

This solution was used as a standard and gave only one spot in both solvent systems A and B above.

RESULTS and DISCUSSION

The significant results are presented in Table 1. The oil content of all samples is within accepted limits. It is, however, a little surprising that the samples contained so many damaged nuts, which suggests that greater care in harvesting is necessary. Five samples had visible evidence of fungal attack on the shells.

The chloroform extract of the meals was first examined for the presence of "aflatoxins" using solvent system A.This system has the advantage for routine work that the constituents are high boiling and that the Rf values from individual chromatograms are not therefore so dependant on external temperatures. As it can be seen from the table, three samples (1,6,7) did not contain fluorescent substances, while with two others (4,5) the amount present was insignificant. This left five samples of which three (3,8,9) contained a fluorescent substance which could be Aflatoxin BI. These five samples were then examined using solvent ssytem B, (the Rf values obtained for this solvent system did vary from external temperature and are all corrected against Aflatoxin BI as an internal standard).

Sample 2 gave no distinct spot with solvent system. Sample 10 although giving a blue fluorescent spot, is obviosly not Aflatoxin BI. With both the solvent systems described Aflatoxin BII has the same Rfvalue as BI. Aflatoxin CI and GII give a green fluorescence.

Of the three samples which could be Aflatoxin BI containing, sample 3 gives with solvent system B a significantly lower Rf value. Further the fluorescense is a light blue and not the characteristic royal blue of Aflatoxin BI. The remaining samples would certainly appear to contain Aflatoxin BI or a closely related substance. It is true that the mean Rf for both samples 8 and 9 is caused by the presence, for example, of other compounds.

Final proof of the presence of aflatoxin BI in these samples would depend on bioassay, see below.

CONCLUSION

Ten samples of groundnuts were examined for the presnce of toxic metabolites of the fungus Aspergillus flavus. Of these samples, two contain compounds which on the present evidence are Aflatoxin Bl.At first sight it seems unlikely that "aflatoxin" should be a problem in Libya. It is recommended, however, that a further study should be carried out, including bioassay, on the 1971 crop. In particular, information should be obtained, on drying and storage procedures.

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